BRNO UNIVERSITY OF TECHNOLOGY
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FACULTY OF CHEMISTRY
FAKULTA CHEMICKÁ

INSTITUTE OF MATERIALS SCIENCE
ÚSTAV CHEMIE MATERIÁLŮ

PREPARATION OF CHITIN FILM
PŘÍPRAVA CHITINOVÝCH FILMŮ

BACHELOR'S THESIS
BAKALÁŘSKÁ PRÁCE

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Bachelor's Thesis Assignment

Number of thesis:	FCH-BAK1321/2018	Academic year: 2018/19
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Student:	**Michaela Debnáriková**	
Study programme:	Chemistry and Chemical Technologies	
Study field:	Chemistry, Technology and Properties of Materials	
Head of thesis:	**Dr. Abdelmohsan Abdellatif, Ph.D.**	

Title of Bachelor's Thesis:

Preparation of chitin film

Bachelor's Thesis assignment:

The aim is to extract and fabricate biofilm from chitin solution using green solvent and study the mechanical and rheological properties of their solution.

Deadline for Bachelor's Thesis delivery: 24. 5. 2019

Bachelor's Thesis is necessary to deliver to a secretary of institute in the number of copies defined by the dean. This assignment is part of Bachelor's Thesis.

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Student	Head of thesis	Head of institute

In Brno, 31. 1. 2019

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prof. Ing. Martin Weiter, Ph.D.
Dean

ABSTRACT

The bachelor thesis deals with the extraction, purification, dissolution and preparation of film based on chitin/ polyvinyl alcohol (PVA). The pure chitin was fully characterized by different techniques like potentiometric titration, FT-IR, TGA, XRD, SEM and solid NMR. The solubility, purity of extracted chitin was investigated and evaluated by measuring the protein percent, rheology, and time need for dissolution. The chitin/PVA films were prepared using different ratio between chitin and PVA solution and film was prepared by casting method. The surface morphology, rheological and mechanical properties of the film were measured and evaluated.

ABSTRAKT

Predložená bakalárska práca sa zaoberá extrakciou, čistením, rozpúšťaním a prípravou filmu na báze chitín/ polyvinylalkoholu (PVA). Čistý chitín bol úplne charakterizovaný rôznymi metódami, ako je potenciometrická titrácia, FT-IR, TGA, XRD, SEM a pevná NMR. Rozpustnosť a čistota extrahovaného chitínu sa skúmala a hodnotila meraním obsahu proteínov, pomocou reológie a skúmaním času potrebného na rozpustenie. Filmy chitín / PVA boli pripravené použitím rôzneho pomeru roztoku chitínu a PVA. Merané a vyhodnotené boli morfológia povrchu, reologické a mechanické vlastnosti filmu.

KEYWORDS

Chitin, Polyvinyl alcohol, film, rheological properties

KĽÚČOVÉ SLOVÁ

Chitin, Polyvinylalkohol, film, reologické vlastnosti

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DECLARATION

I declare that the bachelor thesis has been worked out by myself and that all the quotations from the used literary sources are accurate and complete. The content of the bachelor thesis is the property of the Faculty of Chemistry of Brno University of Technology and all commercial uses are allowed only if approved by both the supervisor and the dean of the Faculty of Chemistry, BUT.

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Student´s signature

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Contents

1 Introduction

Chitin is a free-standing biopolymer and it is a linear polysaccharide composed of (1→4)-β-linked N-acetylglucosamine units. The main sources of chitin are shrimp and crab shells but also insects, molluscs or fungi. The study of chitin and its product is very popular nowadays. The interest in its studies is growing mainly due to its antimicrobial properties, biocompatibility and biodegradation. Gels and various chitin compounds are used in medicine as drug delivers with controlled release, wound dressing materials or in the food industry as food packaging material. By mixing the chitin solution and PVA, it was assumed to obtain unique properties.

The first part of experiments deals with study of chitin structure, solubility in green solvent depending on concentration of chitin. All prepared solutions were characterized rheologically to study viscosities and mechanical properties of chitin solutions. Chitin biofilms were made by mixing 3 % PVA solution with 3 % chitin solutions in different ratios. The viscosities and mechanical properties of prepared solutions of PVA/CH were studied by reological measurements and mechanical test.

2 Theoretical part

2.1 Chitin

Chitin is a natural polysaccharide of major importance. It is synthetized by many living organisms and belongs to the most abundant biopolymers, after cellulose. In the native state, chitin forms structural components in the exoskeleton of arthropods and in the cell walls of fungi and yeast and it is ordered in crystalline microfibrils. It is extremely versatile and can form solid structures on its own as in insect wings or can combine with other components like calcium carbonate to make stronger substances. By this time, the main sources of chitin are crab and shrimp shells. Usually is extracted by acid treatment to remove different salts (e.g. calcium carbonate, phosphate) this step is called demineralization. After demineralization step, the shrimp is treated by the alkaline solution to dissolve proteins (deproteinization step). The detail description is found in chap.3.2.1. [1]

2.1.1 Structure

Chitin is a linear polysaccharide composed of (1→4)-*β*-linked *N*-acetylglucosamine units (figure 1). The chitin chains are connected by forming very strong hydrogen bonds between the -NH groups of one chain and the -C=O groups of the adjacent chain. [2] Chitin structure is a very similar to cellulose, except that an C(2)-hydroxyl group of cellulose is replaced by an acetamide group. This similarity in structure is reflected in the similar positions in the nature, both are acting as structural and defensive materials. The main derivate of chitin is chitosan, which is easily produced by alkaline deacetylation of chitin (deacetylation step).

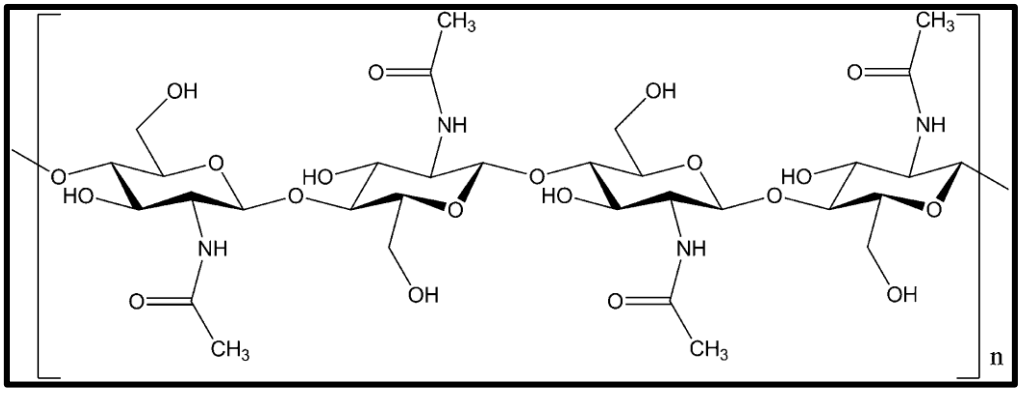

Figure 1. Structure of chitin

2.1.2 Sources and extraction process of chitin

Main sources of chitin are crustaceans, insects, molluscs and fungi. It is very common to obtain chitin from shrimp and crab shells, which are wastes from seafood industries. There are two main ways to obtain chitin: the conventional process and the fermentative process. The first step of conventional process is demineralization, which removes ash content in the crustacean wastes. These ashes are usually composed by carbonates, phosphates and other minerals. For the demineralization is usually realized by using diluted solutions of hydrochloric acid under stirring and ambient temperature. This treatment occurs without degradation of chitin polymeric chains. Other common acids can cause damages in the chitin structure. The second step is deproteinization. In this step is used alkaline medium to remove the protein content. For this treatment is usually used sodium hydroxide under stirring. In this

case temperature varies from 20 to 100 °C and the time period varies from 2h to 72h. High temperature and long-time period can cause depolymerization and deacetylation. The last step to isolate chitin is decolouration/depigmentation. This step is used to remove pigments and odours. For this purpose, are mainly used ethanol, acetone, $KMnO_4$, NaOCl or H_2O_2. All these steps are followed by several consecutive washings until neutral pH. [3]

Chitin can be also obtained from fungi by fermentative process. By fermentation is obtained fungal biomass, which contains glucan complexes with chitin. The biomass is separated from the fermentation media by filtration, after that it is dried and using a few more steps the chitin is extracted. [3]

2.1.3 Properties

Chitin molecules are characterized by high hardness and tendency to associations to form high oriented supramolecular structures. The existence of hydroxyl groups in its structure offers possibility for obtaining of new perspective derivates of chitin based materials.[4] Chitin has various polymorphic forms in nature, it can be found as α-, β-, γ- form. α-chitin is usually insoluble in water, diluted acid and common organic solvents. β-chitin can be swollen in water and dissolved in formic acid. The solubility can be also influenced by molecular weight and DA value. Deacetylated chitin of DA 28 % is soluble in dilute acetic acid, while chitin of DA 49 % with a structure similar to that of β-chitin is water-soluble. Chitin with DA more than 50 % is soluble only in 1-allyl-3-methylimidazolium chloride below 1 wt.% and chitin with lower DA value can be dissolved in ionic liquids. As a good solvent for chitin and chitosan has been shown to be aqueous metal alkali solvent, as a green solvent, that can dissolve polysaccharides at low temperature. [5]

2.1.4 Use of chitin

Thanks to the biodegradability and biocompatibility of chitin and its derivates we find its use in various applications of ordinary life and even in applications that are more complex. It is used for example for water purification, separation of materials or as food additive. In biomedical fields it is used as wound dressings and scaffolds because of their wound healing, antibacterial and anti-inflammatory properties. [6] Due to the film-forming properties and antimicrobial activity of chitin again fungi or bacteria, chitin films replace commonly used food packaging materials as high density polyethylene film, which has disadvantages like fermentation due to the depletion of oxygen and condensation of water which promotes fungal growth. [7]

2.2 Chitosan

Chitosan is poly[*β*-(1→4)-2-amino-2-deoxy-D-glucopyranose] and its structure is shown on figure 2. [8] Chitosan is a linear polysaccharide mainly obtained by deacetylation. It is a polycationic biopolymer and contains amino groups in its structure. It was discovered in 1 859 by Rouget. Due to its unique properties such as antimicrobial character, biocompatibility or bio-adhesivity it is very perspective material. [9, 10, 11]

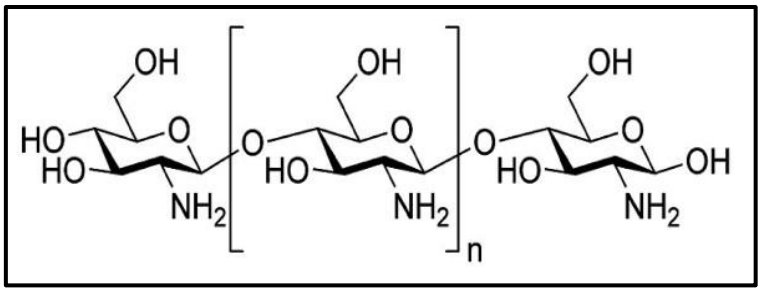

Figure 2. Structure of chitosan [12]

2.2.1 Degree of deacetylation

Deacetylation process is the process in which chitin is converted into chitosan by hydrolysis of its acetamide groups. The scheme of deacetylation of chitin is shown on figure 3. For hydrolysis is used aqueous solution of sodium hydroxide and high temperature. Acetamide groups are hydrolysed and new compound called chitosan contains amino groups. The amino groups in structure of chitin, affects its properties. The quality of chitosan is determinate by various factors, which are molecular weight, degree of deacetylation, the ability of ion exchange and others. For example, high value of the degree of deacetylation means that chitosan contains a lot of amino groups. As consequence, the chitosan solubility and polycationic character is increased. The degree of deacetylation and the molecular weight are dependent on the process of deacetylation. [13]

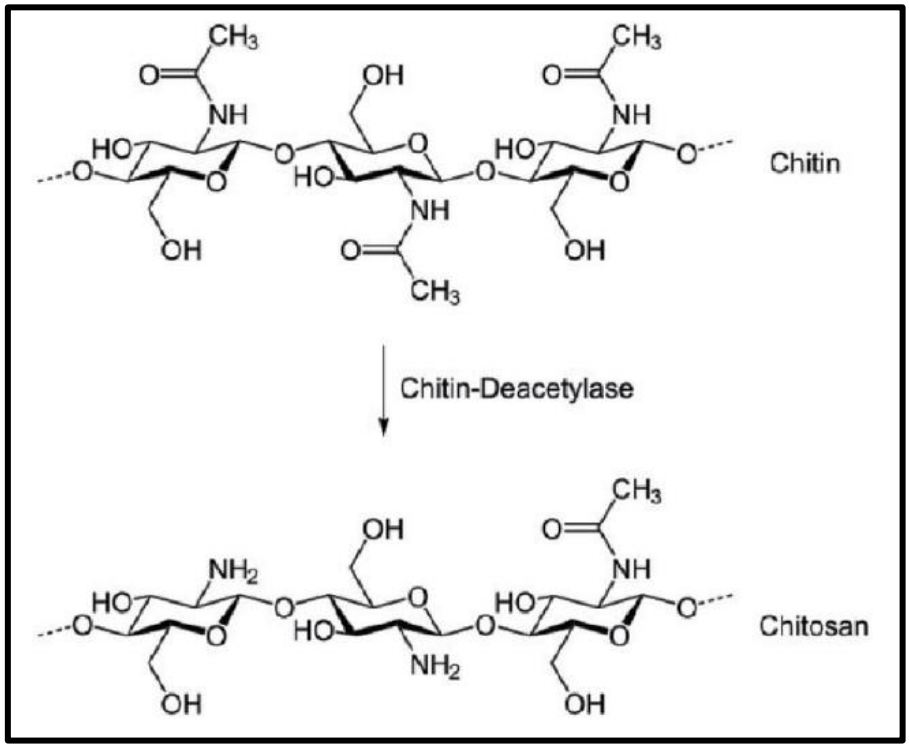

Figure 3. The deacetylation of chitin [14]

The degree of deacetylation can be determined using different analytical methods, such as NMR, FTIR and potentiometric titration. The degree of deacetylation is number of free amino groups present in chitosan's structure. The number of amino groups has an impact on solubility in dilute acid. [13]

2.2.2 Properties of Chitosan

Chitosan properties are dependent on the molecular weight and degree of deacetylation. Important properties of chitosan are polycationic character, film forming, antimicrobial and biocompatibility. Chitosan is a strong base with primary amino groups, so it is soluble in dilute acid solutions below pH 6.0. In the case that pH value is small, H^+ ions can protonate amino groups and chitosan acts as a polycationic biopolymer. When pH value is higher than 6.0, chitosan becomes insoluble. Chitosan is insoluble in sulphuric acid and phosphoric acid. Most commonly used acid medium to dissolve chitosan is hydrochloric acid. [15, 16] Chitosan can form a solid/gel film or membrane with interesting properties. Chitosan is able to form gels due to its muco-adhesive properties and because it can form hydrogen bonds. [17, 18] Chitosan has intrinsic antimicrobial properties, which allow its use in food preservation, pharmacy and biomedicine. This capability is related to polycationic character. Chitosan is able to interact with the microbial cell surface to be specific polycationic part of chitosan molecule interacts with anionic components of cell wall. [19, 20, 21] One of the most important properties of chitosan is its biocompatibility, which allows chitosan to be used in medical applications. [22]

2.3 Gels

Bellow the term gel we understand a macromolecular network with a macroscopic size and elastic properties. They are colloidal dispersion systems in which the dispersed particles are dissolved in dispersed medium and form a macromolecular network. The network can be formed from linear polymer or solution. Connections between macromolecules can be formed by chemical reaction. In this case we speak about covalently crosslinked gel. Other option to connect macromolecular chains, are physical forces, now we speak about physically cross-linked gels. The process of creating gels is called gelation. [23]

2.3.1 Preparation of gels

The gels are made by process called gelation. The moment of appearance of the three-dimensional network and the formation of the gel is called the gelation point. This point can be defined by rheology. During the gelation process, the polymer chains are joined to each other at certain points called nodes. The gel may only be formed when the three main conditions are complied. These conditions are: polymer chains have to be long enough to make stable nodes and complex, the molecule has to have some irregular parts in the structure to avoid crystallization and these irregular parts have to be flexible so they are able to swell. Gels can be created by three different mechanisms: the chemical reaction, the change of physical state, the swelling of xerogel. [23, 24]

2.3.2 Covalently cross-linked gel- chemical network

Covalently cross-linked gels are formed by chemical reaction. These are infinite spatial network structures stabilized by chemical bonds. Structure of the gels made by chemical reaction is very strong. After forming the gel, they are not able to dissolve again, because during the degradation of chemical bonds, those, which have not been created by gelation, can also be degraded. [23, 25]

2.3.3 Physical networks

Physically cross-linked gels are created by formation of nodes by physical forces that allow the agglomeration of polymer chains into these nodes. The macromolecules can be integrated into more than one node region due to their length or the chain, so connected parts are alternated with free parts capable of moving and carrying out a thermal movement. In a contrast to covalent nodes, these physical nodes may be destroyed and re-formed in another arrangement and may also be destroyed by mechanical stress of the gel. The individual gels differ based on the strength and lifetime of the nodes and properties of the nodes influence the behaviour of the gel. Physically cross-linked gels can be divided into weak and strong gels due to its nodes. Strong gels have strong connections and they are elastic. Weak gels, on the other hand, have nodes that can withstand less and therefore are only elastic at low mechanical stress. [23]

2.3.4 Properties of gels

The gels are viscoelastic and therefore, they are able to withstand tensile stress until a certain critical value and behave like an elastically stiff. The critical stress value is determined by the number of nodes and their characteristics. If the forces that are bonding dispersion particles to the network are very weak, it is possible to transfer the gel back to the soil by shaking. The weak bonds between the particles are destroyed by the action of mechanical force. This phenomenon is called rheopexy. If we let the soil stand still, it can form the gel again. This phenomenon is called thixotropy. Thixotropy does not occur in polymer gels whose nodes have different strengths. [25]

2.3.5 Hydrogels

Hydrogels are three-dimensional polymeric networks, which are capable to absorb large amounts of water. They are reversible and physical gels because the main role in forming the network play molecular entanglements and secondary forces like H-bonding, ionic bonding or hydrophobic forces. The ability of hydrogels absorb a large amounts of water is because of presence of hydrophilic groups such $–NH_2$, -COOH, -OH, $-CONH_2$, -CONH- and $-SO_3H$ in the network, capillary effect and osmotic pressure.[26,27,28] When the different polymer chains are cross-linked, the network, which they formed, shows visco-elastic or pure elastic behaviour. The polymer chains can be cross-linked chemically or physically. Chemically cross-linked hydrogels cannot be dissolved they can just absorb water and swell. The physically cross-linked chains are more interesting, because to create them is not needed to use crosslinking agents, which are usually toxic and the have to be removed before using the gel. The methods to create physically cross-linked gels are radical polymerization, chemical reaction of complementary groups, ionic interactions, and crystallization. [29]

Water is the main compound of hydrogels. There three types of water that can be present in hydrogels. First, one is the water in the surface, which is also called free water and can be easily removed in gentle conditions. The absorption of this water happens because of the osmotic pressure; it fills up interstitial spaces and pores in the gel. The second type of water is primarily bounded water. The first interaction, after putting gel in water, happens between polar hydrophilic groups or ions with water and these interactions are hydrogen bonds or ionic

interactions. Due to these interactions, primarily bonded water is absorbed. The outcome of this process is expansion of the network and uncovering of the hydrophobic groups. These hydrophobic groups then interact with water and absorb secondary bonded water, which is also called interstitial water. [30,31] Hydrogels are present in everyday products like contact lenses, hygiene products or in more special applications like drug delivery systems and tissue engineering. [32]

2.4 Poly (vinyl alcohol)

Poly (vinyl alcohol) is a polymer with big interest because of its unique properties, used for various pharmaceutical, biomedical and other applications.

2.4.1 Chemical structure and preparation of PVA

Vinyl alcohol, the monomer of polyvinyl alcohol does not exist in a stable form because it undergoes the rearranging to its tautomer, acetaldehyde. PVA is produced by polymerization of vinyl acetate followed by the hydrolysis. There exist PVA with different degrees of hydrolysis due to the fact, that the hydrolysis reaction can end incomplete and also there may be some acetyl groups in the resulting polyvinyl alcohol. In the figure 4 is shown the picture of PVA and on the figure 5 is shown the preparing reaction of PVA. [33]

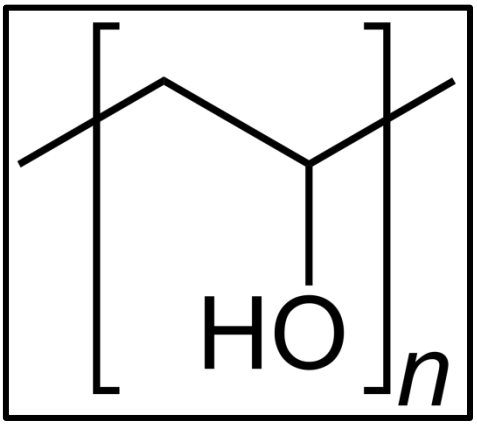

Figure 4. Chemical structure of PVA

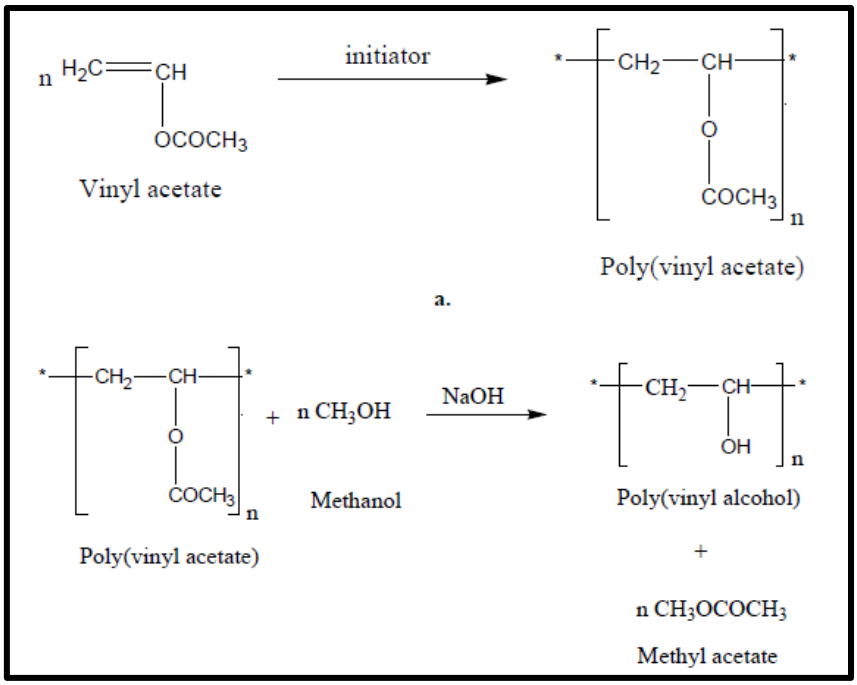

Figure 5. Scheme of PVA preparation [34]

2.4.2 Properties of PVA

Polyvinyl alcohol is a white powdery substance with a crystalline character. Its physical properties are mainly influenced by the degree of polymerization and the degree of hydrolysis there are two main types of PVA available on the market: one with a degree of hydrolysis of about 98 mol.% and second one with the degree of hydrolysis 87–89 mol.%. Both of these types of PVA include products with a polymerization degree of 500–2 500. The solubility of the fully hydrolysed PVA depends on the polymerization degree, the higher the polymerization degree the worse it dissolves. At the same time, the fully hydrolysed PVA provides more viscous solutions for the same polymerization stage than the partially hydrolysed types. PVA is useful in temperatures ranging from 50 to 130 °C, it decomposes above 220 °C. It is resistant to organic acids, but not resistant to inorganic acid and alkali hydroxide solutions. [34]

2.4.3 Use of PVA

Polyvinyl alcohol is used as a protective colloid to provide polymer dispersion stability and it is also used as thickening agent in the manufacture of paints, for the preparation of adhesives, for the impregnation of paper against fats and solvents or in the textile industry. From PVA are made water-soluble and solvent-resistant films. They are used as packaging materials that dissolve in water simultaneously. It is possible to make from PVA also textile fibres. For textile fibres production it is necessary to have PVA with degree of hydrolysis of 99,9 %. The fibres are obtained by extruding a 20 % aqueous PVA solution into an aqueous $(NH4)_2SO_4$ solution or into a mixture of Na_2SO_4 and $ZnSO_4$. In the precipitation bath, the fibres are immediately elongated. The fibres are then dried at 210 or 220 °C. [34]

2.4.4 Properties of PVA gels

A hydrogel made from PVA can be considered as a hydrophilic, cross-linked polymer. It is able to absorbe a big amount of water by swelling and it is not soluble in water. The hydrogels have soft elastic properties and good mechanical properties. The gel properties are dependent on the molecular weight of the polymer, the concentration of the PVA solution the temperature and time of freezing and thawing, and the number of freezing/thawing cycles. These gels are non-toxic, bioadhesive, easily processed and also non-carcinogenic. PVA is able to simulate a natural tissue and can be easily accepted for the body. [33]

2.4.5 Use of PVA gels

PVA gels have found application as contact lenses, in drug delivery applications and also in engineering devices. It can be also used in textile industry as a sizing and finishing agent. It is allowed for use as an indirect food additive in products which are in contact with food, so it is used for example as a diluent in colour additive mixtures for colouring shell eggs. The membranes of PVA are used in a broad range of applications because the membrane can control the permeation rate of a chemical species through the membrane. In drug delivery this property is very important because it is possible to control the permeation rate of a drug from a reservoir to the body. [33]

2.5 Rheology

The rheology studies the flow. It deals with deformations of materials due to the external forces and flow properties of substances in liquid state. The flow can be considered as a special kind of deformation, which is particularly noticeable for materials in liquid state. The flow, or viscous deformation, is characterized by the fact that when the external forces operate, the deformation continues to increase, the rate of deformation growth is directly proportional to the acting force. [35] The deformations of materials can be elastic or non-elastic. If we apply on the material forces, that are smaller than a critical (tensile) stress value, after a force application, the material comes back to his original state. This is how elastic deformation works, which is mainly characteristics for materials in solid state. If we apply on the materials forces that are higher than a critical (tensile) stress value, after a force application, the material does not come back to the original state, but it loses its elasticity and flow. This type of deformation is called non-elastic. In between these two extremes, we can find materials, whose response on deformation forces depends on the duration of force application. These materials are called viscoelastic. [36]

2.5.1 Hook's law

Hook's law deals with the deformation of ideal elastic substances in solid state, in which we cannot observe dissipation of energy. All the energy that is consumed during the stress of the material is re-used, after its release. The behaviour of such materials is similar to the behaviour of the spring and the deformation is reversible or it is also called elastic. The tensile stress σ causes elongation of the body. Hook's law can be expressed by the equation:

$$\sigma = G_e \cdot \frac{\Delta l}{l} = G_e \cdot \varepsilon. \qquad (1)$$

Where, $\varepsilon = \frac{\Delta l}{l}$ represents relative extension or deformation and G_e represents Young's modulus of elasticity. [37]

2.5.2 Rheological division of substances

Newton's law deals with deformation of fluids with ideal viscosity properties. Fluids, which are acting as newton's law describes are called *Newtonian fluids*. These are mostly pure liquids and solutions of low-molecular weight substances. In this case, during deformation, their molecules do not influence each other. We can observe just small interactions between particles. The increasing tensile stress does not influence the change of viscosity of the liquid substance, it just linearly dependents on the shear rate. [38, 39]

Newton's law for *Newtonian fluids* is represented by equation:

$$\sigma_{xy} = \eta \cdot \gamma_{xy}, \tag{2}$$

Where, σ_{xy} stays for shear stress, η represents dynamical viscosity and γ_{xy} is the shear rate. From this equation results that viscosity of *Newtonian fluids* does not change with increase of the tensile stress. Substances on which we cannot apply this law are called *non-Newtonian fluids*. [40] Non-Newtonian fluids are colloidal dispersion systems and heterogeneous dispersion system. These are polymer solutions, salts, suspensions or pastes. It is not possible to determine a single viscosity value for these solutions, which would have constant value across the full range of possible values of tensile stress and due to this non-constant viscosity, substances have specific mechanical properties. [35]

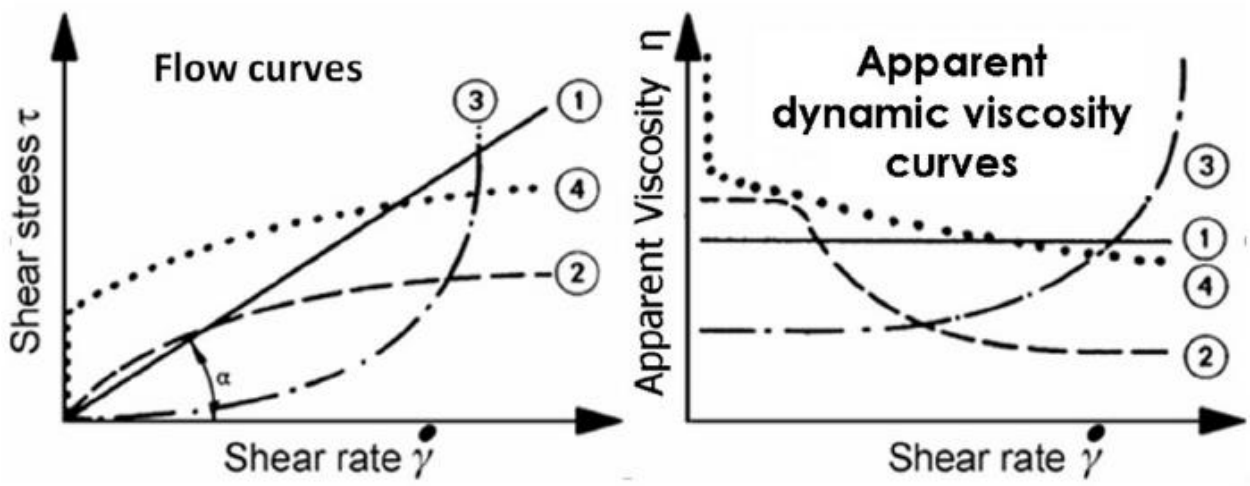

Figure 6. Flow behaviour of Newtonian (1) and non-Newtonian (2,3,4) fluids; (2)- pseudoplastic liquid, (3)- dilatant liquid, (4)- plastic liquid [41]

2.5.3 Types of non-Newtonian fluids

Pseudoplastic fluids- their apparent viscosity decreases with increasing the gradient of velocity. We can observe the change of the structure right after application of the shear stress. Pseudoplastic behaviour is in a practical area desirable property, because it reduces energy needed for mixing. [36,39]

Dilatant fluids- these fluids increase their relative viscosity by increasing the shear rate. For technological processes, this property is not desirable, so usually is influenced for example by the change of composition. [36,41]

Plastic fluids- they have a plastic component of deformation. Their flow occurs after the flow limit is exceeded τ_k, which is a certain limit value of the shear stress. In this category of non-Newtonian fluids we can include Bingham's plastic fluids. They are fluids with a plastic component of deformation, but below the flow limit value they also have elastic component of deformation. [41]

Graphical representation of these types of non-Newtonian fluids we can see on the figure 7.

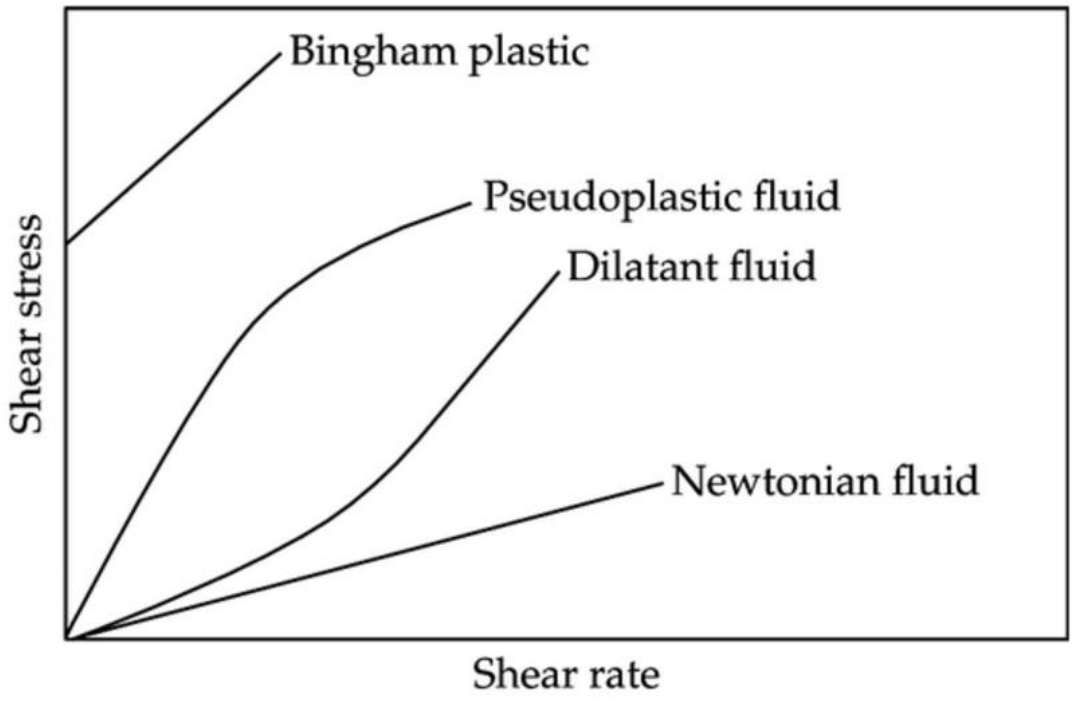

Figure 7. Comparison of different types of non-Newtonian fluids and Newtonian fluids [42]

2.5.4 Thixotropy and Rheopexy

Thixotropy and rheopexy are other phenomenon of the flow behaviour of non-Newtonian fluids. The internal structure changes because of time effect of deformation and that influences the fluid's macroscopic behaviour. For thixotropic liquids, the viscosity decreases with the time of mechanical deformation. For rheopexic liquids viscosity increases, with the time of stress application and so prevents further deformations. [41]

2.5.5 Viscoelasticity

In the practical environment we work with many real substances, which with their properties act as a partially stiff elastic body, but also a viscous liquid. These are not ideal substances, so it is not possible to apply Newton's law on them. These substances are designated as viscoelastic. These are usually polymers, which behavior is dependent on the temperature. It is possible to describe these fluids with two types of modulus. One of them is the elastic module for solids, referred to as *G'* and it is related to ability of the body to retain elastic energy. It creates the real constituent of the complex modulus (referred to as $G^{*)}$ and it refers to the energy stored in the material during the stress cycle. The viscous fluid modulus, referred to as *G''* and it is related with an irreversible change of deformation energy, thus represents the loss of the energy during stress cycle. It creates the imaginary component of the complex modulus G^*. From the equation 3 results, that a complex modulus includes both components that express the rheological behaviour of real viscoelastic substances.

$$G^* = G' + iG'' \tag{3}$$

From these variables we can obtain equation for loss angle, which express ratio between loss and storage modulus (equation 4):

$$tan\delta = \frac{G''}{G'} \tag{4}$$

Due to the size of loss angle can be deduced character of the liquid's behaviour. If the material has both, elastic and viscous component the viscoelastic behaviour can be observed. In the case that $\delta > 45°$ material has a viscous character, in the other case when $\delta < 45°$ the material acts as elastic. [43,44]

2.5.6 Rheology of the gels

In the figure 8 is shown a gelation curve, where axis x stands for time and axis y for elastic and viscous modulus. For the samples that undergo crosslinking, during the measurement are tests performed at constant amplitudes of deformation, from the linear area of viscoelasticity, especially for the measurement of time dependencies. In the time point called gelation start can be observed that values of loss and storage modulus are increasing, until asymptotically converge to a constant value. Before forming gel, the sample is found in the sol stage and shows viscous behaviour and $G' < G''$. The point, in which the sample is transformed from sol to gel is called gelation point and it is a fact that in this point $G' = G''$. From the moment where the elastic modulus acquires higher values, sample exists in the gel state and $G' > G''$. By the measurement of elastic and viscous modulus we can determinate the cross-over point, which is the point where the curve of elastic modulus meets the curve of viscous modulus. [43,44]

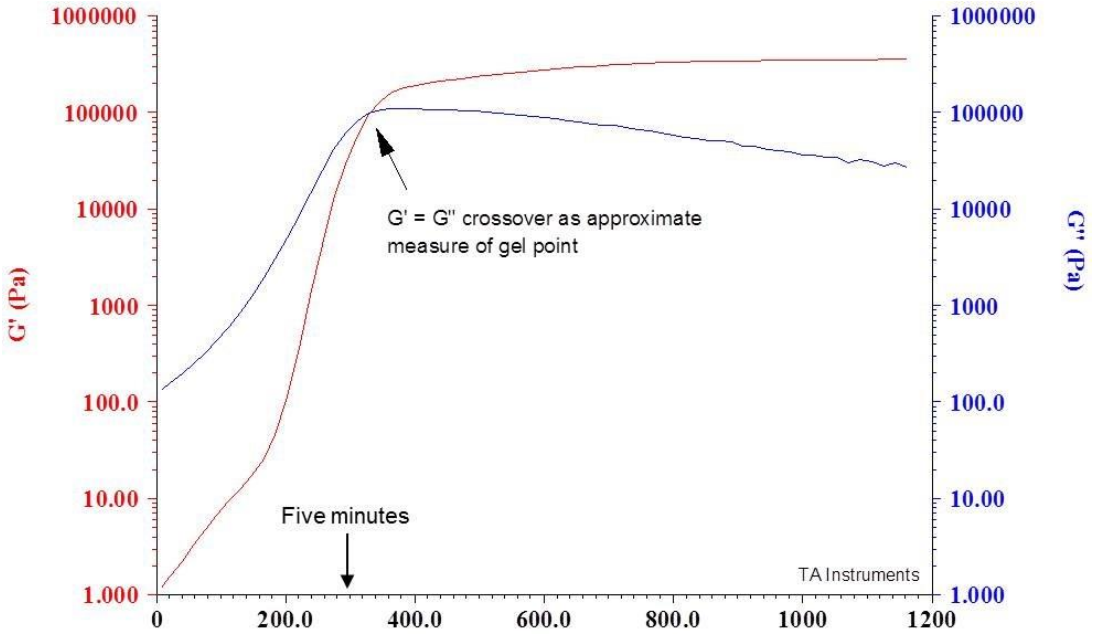

Figure 8. The graphical representation of the cross point of storage and loss modulus [45]

2.5.7 Measuring systems

There are many types of analytical methods for measuring the viscosity. Rotary viscometer (rheometer) is composed of two basic parts, which are rotor and stator. The stator is the static part and rotor is the part that is moving. The rotor rotates with a constant angular velocity. The rheometers can have different tool geometries that are used depending on the viscosity of measured material. The types of geometries are described on figure 9 and figure 10. For gel samples is advantageous to use parallel plate geometry. The main advantage is the small amount of sample that is needed. Concentric cylinder and double gap geometries can be used to measure low-viscosity substances and Newtonian fluids. [46]

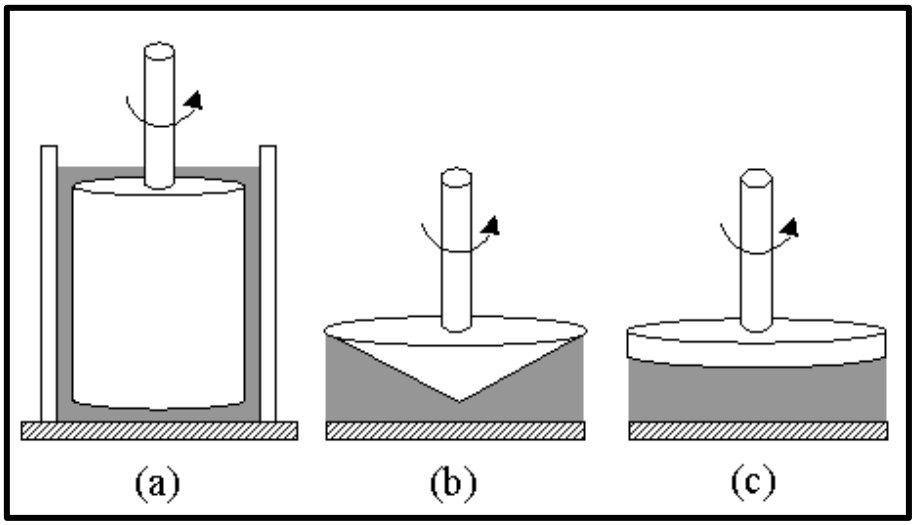

Figure 9. Basic tool geometries for the rotational rheometer: a) concentric cylinder, b) cone and plate, c) parallel plate [46]

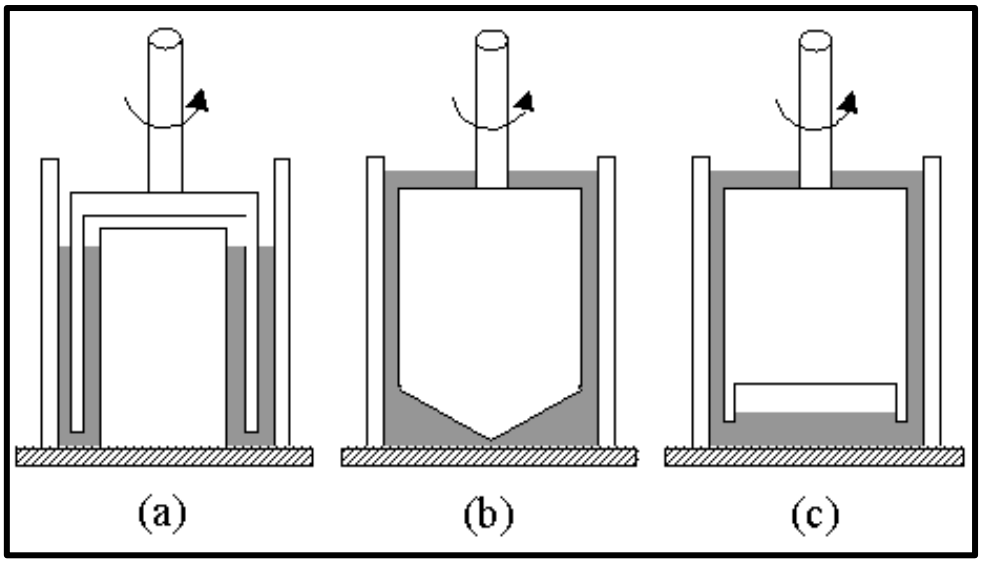

Figure 10. Alternative cylindrical tool designs in cut-away view: a) double gap, b) cone and plate at the bottom, c) hollow cavity at the bottom to trap air [46]

3 Experimental part

3.1 Materials

- Sodium hydroxide, NaOH, M_w = 39,997 g/mol, CAS: 1310-73-2, Lach-Ner,s.r.o
- Hydrochloric acid, HCl, M_w = 36,46 g/mol, CAS: 7647-01-0, Lach-Ner,s.r.o
- Urea, CH_4N_2O, M_w = 60,06 g/mol, CAS: 57-13-6, Lach-Ner,s.r.o
- Ninhydrin, $C_9H_6O_4$, M_w = 178,14 g/mol, CAS: 485-47-2, Sigma Aldrich
- Hydrindantin, $C_{18}H_{10}O_6$, M_w = 322,268 g/mol, CAS: 5103-42-4, Sigma Aldrich
- Ethanol, CH_3CH_2OH, M_w = 46,07 g/mol, CAS: 64-17-5, Sigma Aldrich
- Polyvinyl Alcohol, $(C_2H_4O)_x$, average M_w = 70 KDa, 99+% hydrolysed, CAS: 9002-89-5, Sigma Aldrich

3.2 Methods

3.2.1 Chitin extraction and purification

Extraction of chitin was done using shrimp shell as source of chitin and purification of chitin consist of two important steps (demineralization and deproteinization). Both processes are described below. Demineralization is the first step. To do so, 5 % solution of hydrochloric acid have been used for 2h at ambient temperature (approx. 21 °C). After 2h chitin was washed with demineralized water until neutral pH. Subsequently chitin was washed with ethanol and dried at 40 °C. The entire process has been repeated for five times. For deproteinization was used 5 % solution of sodium hydroxide. The solution of sodium hydroxide with chitin was heated to 90 °C for 5h. After 5h solution was washed with demineralized water until neutral pH. Then chitin was washed with ethanol and put into dryer set on 40 °C until next day (approx. 14h). This process was repeated for 5 times.

3.2.2 Preparation of chitin solutions using green solvent

Chitin solutions were prepared using green solvent. Green solvent is a metal alkaline solution of sodium hydroxide and urea. Mixture of 12 % NaOH solution and 8 % urea solution was left in the freezer until frozen (-15 °C). After that certain amount of chitin was added into the solution. The composition of the samples and their labels are given in table 1. The solution was blended every two hours. All prepared solutions were stored in the freezer at -25 °C.

Table 1. The composition of the prepared gels

Signature	NaOH [hm%]	urea [hm%]	chitin [hm%]
CH 1	12	8	0,5
CH 2	12	8	1
CH 3	12	8	2
CH 4	12	8	3
CH 5	12	8	3,5
CH 6	12	8	4
CH 7	12	8	4,25
CH 8	12	8	4,5
CH 9	12	8	5

3.2.3 Preparation of chitin solutions with PVA

Solutions of PVA and chitin were prepared by mixing solution of PVA and chitin. The ratio in which the solutions were prepared is given in table 2.

Table 2. Composition of chitin and PVA solutions

Signature:	CH [g]	PVA [g]
CH/PVA 0/100	0	25
CH/PVA 10/90	2,50	22,50
CH/PVA 20/80	5,00	20,00
CH/PVA 25/75	6,25	18,75
CH/PVA 40/60	10,0	15,00

3.2.4 Film preparation

Net chitin films were prepared by using different concentration of chitin (3 and 5 %) by casting method. The degassed solution of chitin was placed on Petri dish and kept on air until it was completely dried. After that the salts which appeared on the surface of chitin film were washed with water and ethanol. The films were dried in oven set on the 40 °C for 2 days. The degassed solution of chitin and PVA was placed on Petri dish to evenly cover its entire surface. Solution was left to dry on laboratory temperature on air. After the film was formed, the salts created on the surface of film were removed using water. The films were dried in the oven set to 40 °C, between two heavy slabs to get compact film with constant thickness.

3.3 Characterization of pure chitin and films

3.3.1 Protein determination

Ninhydrin test was used to determinate protein content in chitin. During the process, protein connected with chitin is hydrolysed by sodium hydroxide treatment and the concentration of ninhydrin-positive substances in the hydrolysate is determined colourimetrically. 0,3 g of chitin sample is added to 50 cm^3 of 10 M sodium hydroxide in a boiling flask. The boiling flask is heated at 120 °C for 60 minutes. After this time, the mixture is cooled rapidly and neutralized with concentrated hydrochloric acid in ice bath. After neutralization, solution is filtered. The filtrate and washings are mixed together and water is added to prepare 150 cm^3 of sample solution. In a test tube, 0,5 cm^3 of the sample solution, 5 cm^3 of acetate buffer with pH 5 and 5 cm^3 of ninhydrin-hydrantin solution are mixed together, by shaking. After that, test tube is covered with aluminium foil and kept in the boiling water for 10 minutes. After rapid cooling, the absorbance is measured at 564 nm. Amount of protein present in chitin is calculated by equation 5:

$$P(\%) = 2{,}37 \cdot \left(\frac{A_{564}}{W}\right) \tag{5}$$

Where A_{564} is the absorbance at 564 nm and W is the weight of chitin sample in grams.

3.3.2 Potentiometric titration

Potentiometric titration was used to determinate degree of deacetylation of chitin. Chitin sample (0,2 g) was added to 20 cm^3 0,1M HCl solution. This solution was titrated using standardized 0,1M solution sodium hydroxide under constant stirring. pH was measured using a pH-meter. For visual determination of equivalent point was used methyl orange. Potentiometric titrations were made three times. The degree of deacetylation was then calculated from two equivalent points using equation 6:

$$DD = 161 \cdot 10^{-3} \cdot (y - x) \cdot \left(\frac{M}{w}\right) \cdot 100 \tag{6}$$

where number 161 represents the molar mass of the monometric unit of fully deacetylated chitosan ($g \cdot mol^{-1}$), y and x are the second and first equivalent points (cm^3), M stays for concentration of NaOH standardized solution ($mol \cdot dm^{-3}$) and w is the chitosan weight (g).

3.3.3 FT-IR

FT-IR (ATR) spectrum were recorded for chitin with different time of alkaline treatment for pure PVA for PVA film for chitin films of different concentrations and for films made from mixture of PVA and chitin. The spectrums were recorded using a Bruker Tensor 27 IR spectrometer, by attenuated reflection on a diamond crystal with an angle of 45 degrees, at a resolution of 4 cm^{-1}.

3.3.4 TGA

Thermal degradation of chitin, PVA and the films was performed using TGA Q 500(TA Instruments, U.S.A.) in the temperature range of 25–800 °C (298,15–1073,15 K) at a heating range of 10 °C/min in nitrogen atmosphere.

3.3.5 X-ray diffraction (XRD)

XRD was measured at 3 kW diffractometer Smart lab (Rigaku) using Cu Kα radiation (λ = 1.54 Å) and detector Dtex Ultra with Bragg-Brentano geometry. Diffraction angle 2-Theta (XRD) was measured in range from 5° to 60° with step size 0.02° at speed 4°/min. Generator was operated at current 30 mA and voltage 40 kV.

3.3.6 Scanning electron microscope SEM

SEM Mira3 XM (Mira, Czech Republic) was used for imaging of morphology of used materials to prepare films as well as composite biofilms. In order to achieve better resolution and prevent to overcharging the samples were coated with the thin conductive metallic layer of Au alloy (thickness 20 nm). Imaging of morphology was obtained at different magnifications in resolution regime with 10 kV, using secondary emission detector.

3.3.7 Rheology

The chitin solutions with concentration range from 0,5 % to 5 % and solutions of chitin and PVA were characterized for their rheological properties using steady shear and dynamic oscillatory tests. Rheological measurements were performed on an ARES-G2 rheometer (TA Instruments, U.S.A.). A cone and plate geometry was used for monitoring the steady-state shear flow and dynamic rheology. The solution was set on desired temperature directly on rheometer. The first measurement was a deformation amplitude test. At a constant frequency of 1 Hz the dependence of elastic and viscous modulus on the varying amplitude of the deformation was determined. At the same time, optimal value of the strain amplitude was set to 1 %. Consequently, dependence of elastic and viscous modules on oscillation frequencies was obtained by frequency tests. The measurement took place at the selected value of the sensor oscillation in the range of 0,1–20 Hz. The steady shear viscosities of solutions were determined rheometrically at 20 °C, angular frequency 1 rad·s^{-1}, rate 0–20 s^{-1}. Also the dependence of viscosity of individual samples on temperature was observed, with the temperature range used for chitin solutions from 5–50 °C (5 °C/min), strain amplitude was set to 1 % and frequency to 1 Hz. TRIOS software by TA Instruments and MS Excel program was used for evaluation of acquired data. The steady shear viscosities of solutions were determined rheometrically at 20 °C, angular frequency 1 rad·s^{-1}, rate 0–20 s^{-1}.

3.3.8 Mechanical tests

Mechanical tests were performed to study mechanical properties of prepared films. Three films were selected for this measurement, net PVA film, the film with 90 % of PVA and 10 % of chitin and the film with 80 % of PVA and 20 % of chitin. Tensile test was performed on ZWICK Roell Z 010. The test specimen was clamped so that the longitudinal axis of the specimen was coincident with the jaw axis. The clamping is shown figure 11. The samples were measured at a strain rate of 1 mm·min^{-1} at laboratory temperature and the test was finished after sample breakage. Every film was measured for four times. For

each sample the thickness was determined in three places and the values obtained were subsequently averaged.

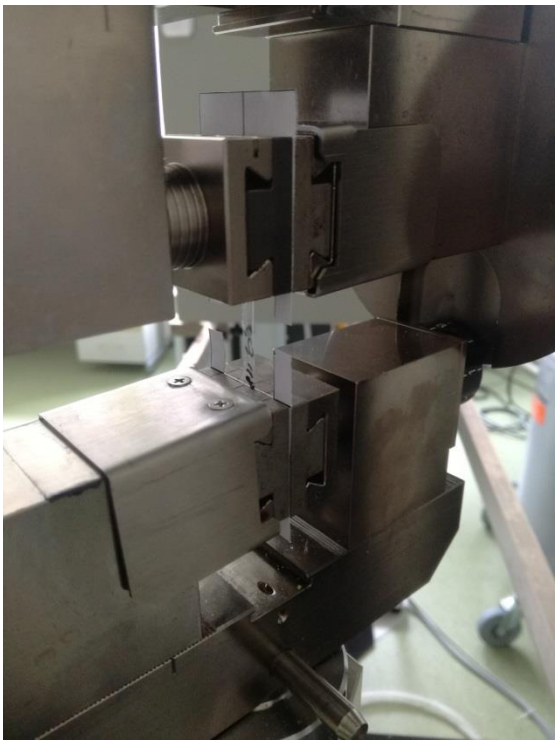

Figure 11. The detail of clamping sample in jaws of measuring device

4 Results and discussion

4.1 Extraction of chitin

Extraction of chitin using acid and base treatments from shrimp shell were investigated and evaluated. In shrimp shell, chitin is found as a part of a complex based on proteins and minerals as calcium carbonate and calcium phosphate deposit to form the rigid shell. Thus, the isolation of chitin from Atlantic shrimp shell is summarized in two major steps: removal of calcium salts in demineralization step using diluted hydrochloric acid and second step: removal of proteins in deproteinization process using sodium hydroxide. In demineralization step, shrimp shell contains about 10–25 % of minerals and the huge amount of these minerals is calcium carbonate and calcium phosphate. Hydrochloric acid was used as a reagent in demineralization step in order to remove the minerals (as calcium carbonate) from the shell. In this process, minerals were hydrolysed into highly water-soluble salts which could be separated by filtration and washing with deionized water for several times. In deproteinization step, the chemical treatment is used to destroy the covalent chemical bonds between the chitin-protein complexes (proteins are bound by covalent bonds to the chitin through aspartyl or histidyl residues or both forming stable complexes such as glycoproteins. Alkaline solution from sodium hydroxide is used to remove the proteins, lipids and pigments from the crawfish shell. The removing of minerals is done using 5 % HCl at room temperature for 10 hours and proteins are removed using 5 % NaOH at 90 °C for 24 h.

4.2 Characterization of chitin

4.2.1 Degree of deacetylation

Method of potentiometric titration was used to determine degree of deacetylation of used chitin. The degree of deacetylation that was procured was 7,8 %. For graphical representation of titration was chosen one representative measurement. In the figure 12 can be seen whole titration curve and in the figure 13 can be seen the selected part that displays the two equivalence points used for the calculation. The equivalence points are highlighted by red colour. Titration measurements are carried out three times and we the degree of deacetylation was calculated as an average of these three measurements.

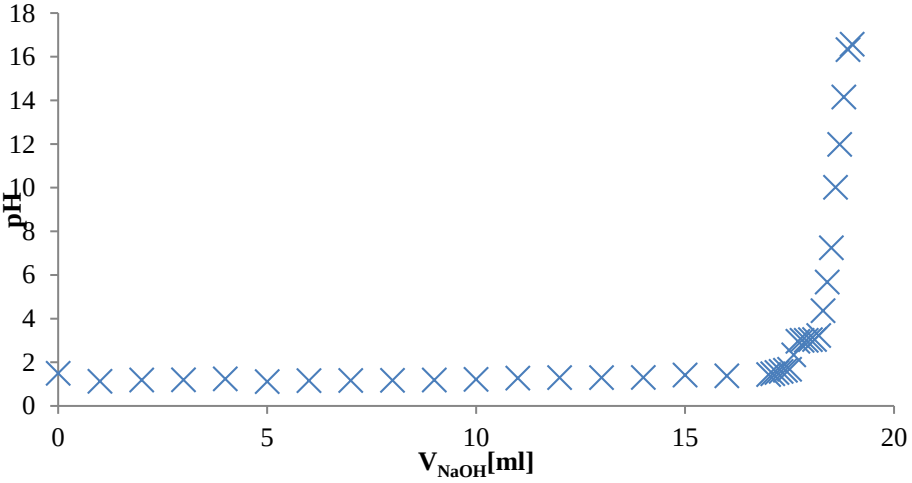

Figure 12. Titration curve of chitin sample

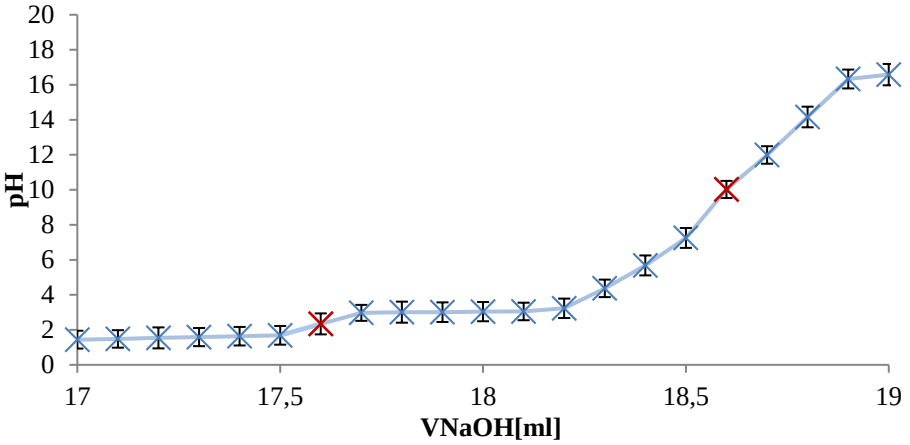

Figure 13. Selected area of the titration curve

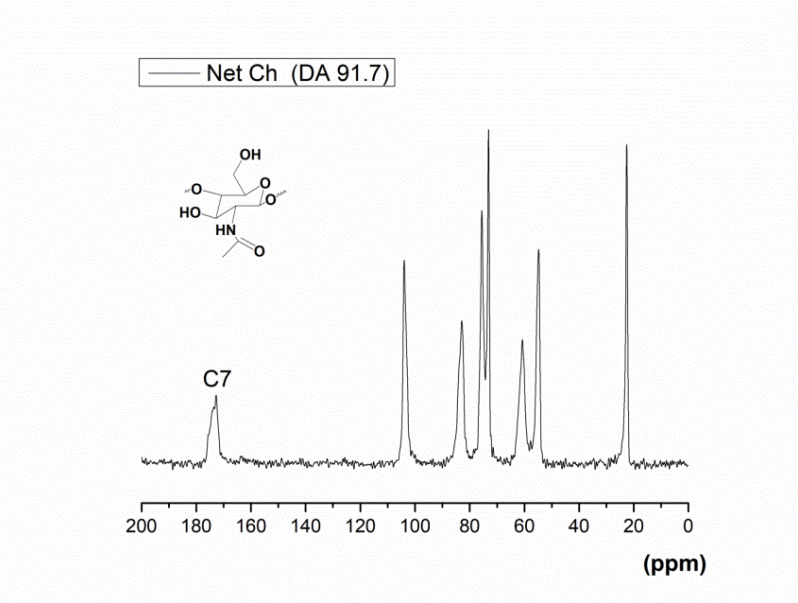

Figure 14. Solid state NMR of chitin

The degree of deacetylation was also acquired from solid state NMR to compare the results. The spectra of solid NMR, is shown on figure 14. On this figure can be seen degree of acetylation which is 91,7 % what means that degree of deacetylation is 8,3 %. The results are also collected in table 3.

The results obtained by potentiometric titration and NMR are shown in table 3, where can be also seen the difference between these two results which is 6,87 %. There is not a significant difference between method used for determination the degree of deacetylation of chitin.

Table 3. Results of DDA% of both methods

Potentiometric titration	NMR	Difference [%]
7,73	8,30	6,87

The ninhydrin test was performed to determine the amount of residual proteins in pure chitin, since the protein content significantly affects the properties of chitin and on the ability of chitin to form a clear solution. From figure 15, the residual of proteins are decreased by increase the time treatment using sodium hydroxide (5 %; 90 °C) from 25 to 55 h and residual of protein (%) is less than 0,05 % after 55 h.

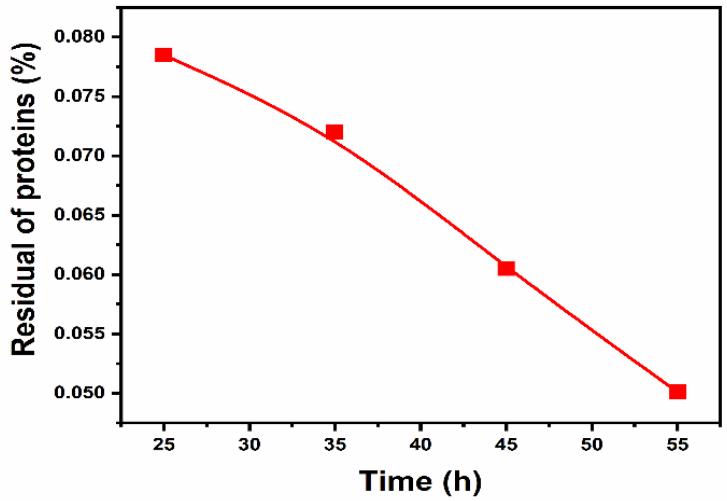

Figure 15. Effect of time treatment using sodium hydroxide on residual of protein after deproteinization step.

4.2.2 FTIR-ATR

Figure 16 shows the effect of time using sodium hydroxide (5 %; 90 °C) on the structure of chitin polymer. The spectra were recorded for samples every 5 hours of alkaline treatment to see which changes take a place in chitin structure during the treatment. There are several characteristic absorption bands for chitosan such as C-O stretching can be observed at frequency of 1 090–1 024 cm^{-1}. The –CH vibration can be seen at 1 152 cm^{-1}, at 1 255 cm^{-1} it is possible to see C-O-C bands. At 1 348 cm^{-1} can be observed $–CH_3$ symmetrical deformation, –C=O and amide band –CONH- is present at 1 639 and 1 561 cm^{-1}. The stretching vibrations of $–NH_2$, -OH groups and hydrogen bonds that overlap each other can be observed at 3 438 cm^{-1}. The presence of carbonyl group indicated by the peak at 1 639 cm^{-1} is attributable to low degree of deacetylation of chitin. In all spectrums can be seen a significant peek at 1 561 cm^{-1} which indicates amide absorption band. In the figure 16 we can see differences in the intensity of carbonyl group at the 1 655 cm^{-1} and the peak of amide group at 3 449 cm^{-1}. In the figure can be observed that the peak in the range 3 400 to 3 100 cm^{-1} became wider with increasing DDA percentage. We can conclude that all appeared peaks related to pure chitin only and there are any peaks related to proteins.

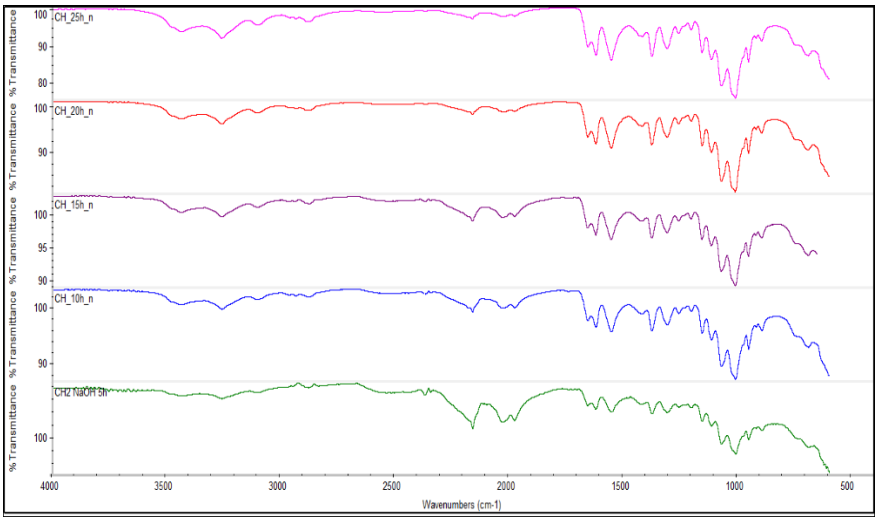

Figure 16. FTIR spectra of chitin

4.2.3 TGA

The all thermogravimetric date were recorded under the conditions that are mentioned before. Figure 17 shows mass loss vs. temperature curves of pure chitin, chitin after alkaline treatment and 3 % chitin film. The thermograms showed only one step of degradation. The degradation of pure chitin (CH_HCl) occurred in one single step in temperature range from 320 °C to 418 °C with the peak temperature at 380 °C. The degradation of chitin after alkaline treatment (CH_NaOH) occurred in temperature range from 312 °C to 411 °C with the peak temperature at 370 °C. It can be seen on the graphical representation that the alkaline treatment did not affect the temperature stability of chitin samples. The degradation of chitin film also occurred in one single step in the range of temperature from 270 °C to 340 °C, with the peak temperature at 307 °C. The thermograms, also shows that degradation of whole sample of chitin film takes more time than the chitin samples. For graphical representation area between 0–600 °C was chosen, because in the higher temperature the curves were not clear, due to the possible presence of impurities in measuring system.

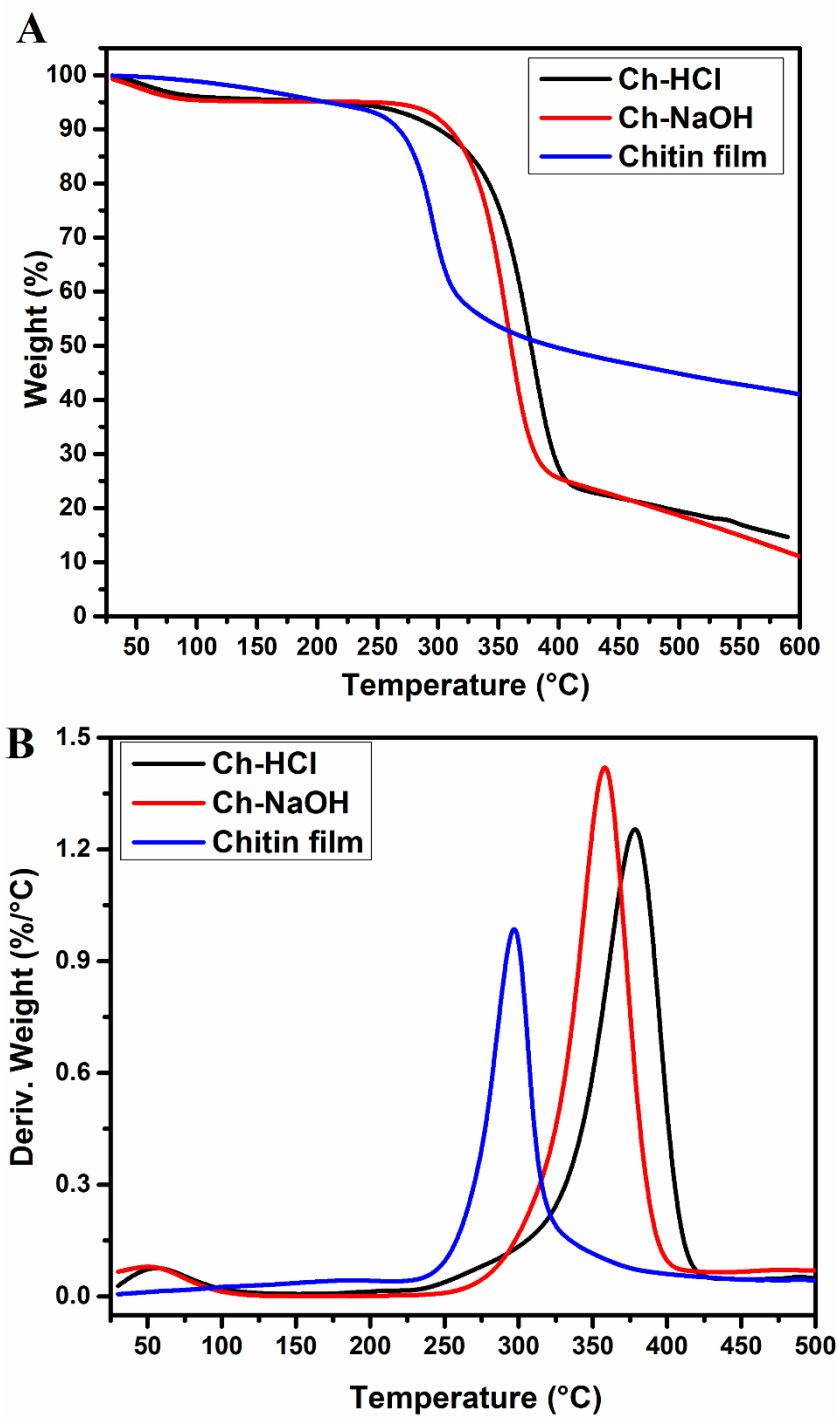

Figure 17. The thermograms of pure chitin, chitin after alkaline treatment and 3% chitin film after dissolution in green solvent.

4.2.4 SEM

Chitin under secondary electrons reveals fibrous like texture. Fibres are without preferred orientation packed into nets. Singular nets form massive layer without internal texture.

Figures 18–20 show the SEM of net shrimp shell, and shells after treatment with acid and base, respectively.

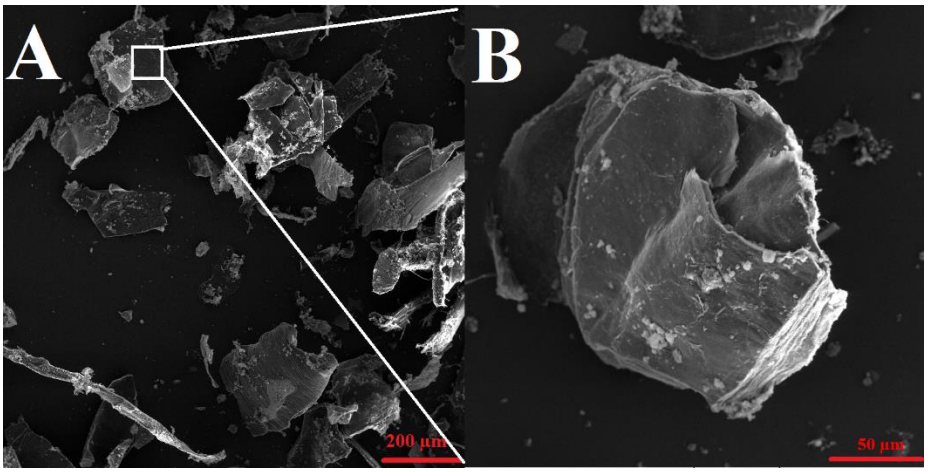

Figure 18. Representative SEM of net shrimp shell using different magnification.

Figure 18 shows the SEM of net shrimp shell before any acid or base treatments. As shown in figure 18, hereogenious sizes of shrimp shell with some impurites on the surface of shells.

There no any fibrilis structure appeared before acid/ base treatments. This confirmed strong interactions in matrix between chitin/protein/minerals composite. From the figure 19 is clearly visible presence of the rounded desication pores structure and pore sizes up to 0.7 µm in diameter. Origin of chanels is the most probably due to sample preparation process. The pores appeared in surface of shrimp shell after acid treatment may be due to removal of minerals (Ca, Pd, etc.,) from the matrix chitin/protein/mineral in shrimp shell surface. Fibrilis structre of chitin start to appeared after acid treatment.

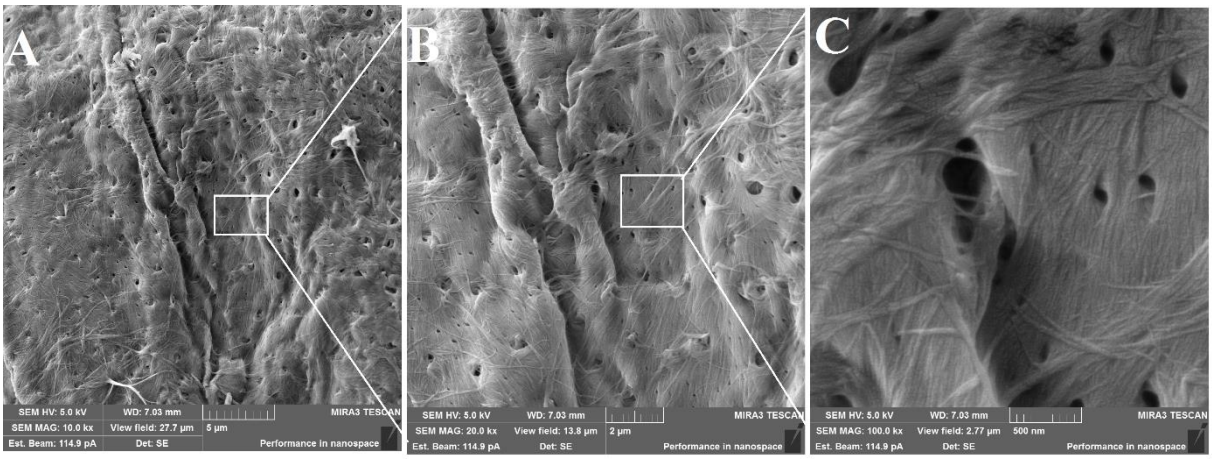

Figure 19. SEM pictures of chitin surface after acid treatment at different magnifications.

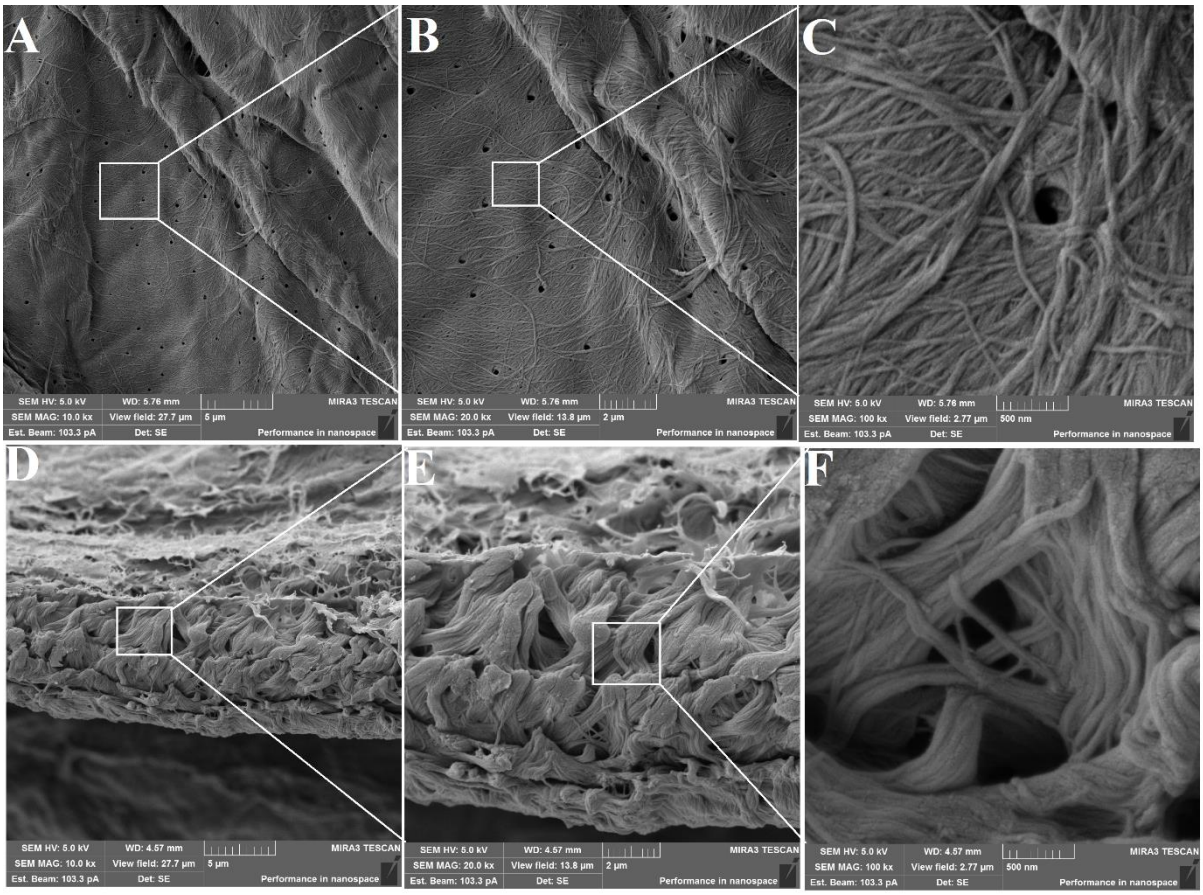

Figure 20. SEM pictures of chitin after alkaline treatment. (A-C) surface morphology of chitin after acid and based treatment, respectively. (D-F) cross-section of chitin microparticles after acid, base treatments at different magnifications.

SEM of shrimp shells after acid and base treatments is shown in figure 20. As shown, more clear and smooth surface of chitin with highly fibrils structure appeared after base treatment using sodium hydroxide time. The fibrils structure more bundle each other in groups of monofilaments. Figure 20 shows the SEM of chitin microparticles after acid and based treatment, respectively. Clear, smooth with highly compared with net shrimp shells. More fibrils structure appeared with small single monofilaments and more bundling form structure with size of monofilaments about 30–50 nm (diameter). From surface and cross-section of pure chitin (figure 20) we can conclude that after acid and base treatment we can prepare pure chitin with fibrils structure that can be used in different application especially for medical purposes.

4.2.5 Preparation of solutions

As a part of this work was monitored dependence of concentration of chitin on solubility in NaOH/urea solvent. Eight different concentrations of chitin were prepared by the same way described in chap. 3.2.2. Their solubility and stability were controlled every 24 hours. The solubility and stability depend on concentration of chitin. As the first was made solution with lowest chitin concentration and also it was the fastest in being stable. The higher concentrations were stable after one month of being kept in freezer with temperature range of -20 to -30 °C. The comparison between first day of solution, unstable state of solution and

stable solution is shown on figure 22. Graphical representation of time needed to prepare stable solutions of different concentrations is showed on figure 21.

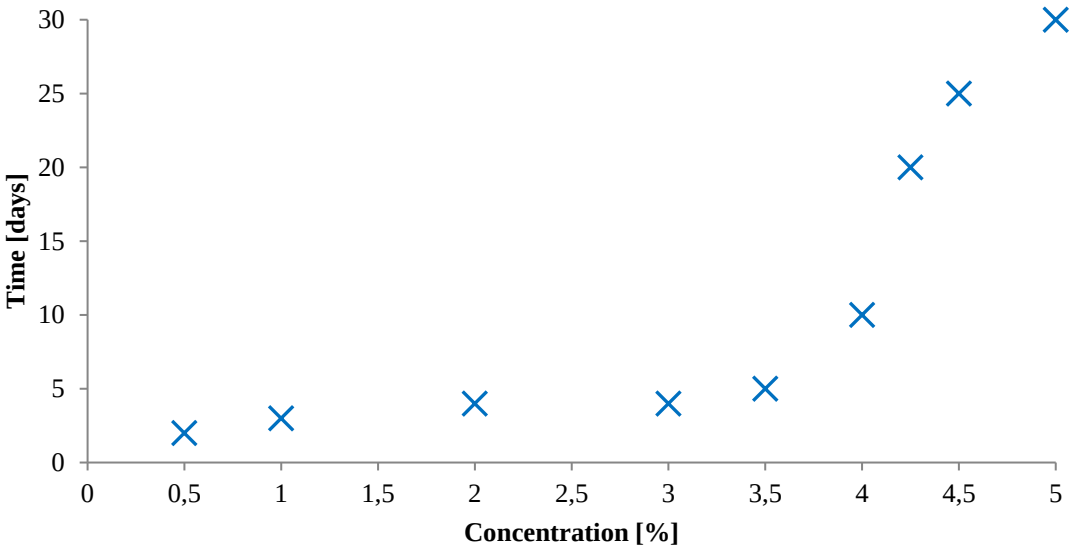

Figure 21. Time duration of preparing solutions of different concentrations

On the figure 22 can be observed that after putting together chitin and alkaline solution is created heterogeneous solution where can be markedly seen fibres of chitin. After some time in freezer, the time needed depends on the concentration of chitin, the fibres start to be transparent and hardly seen by an eye. On the second picture (B) can be observed the clear and stable solution of chitin without any non-dissolved particles inside. In the last picture (C) is shown unstable solution of chitin. On this picture can be observed bleary white solution with visible particles. The particles appear after a certain time on laboratory temperature, this time also depends on concentration of chitin in the solution.

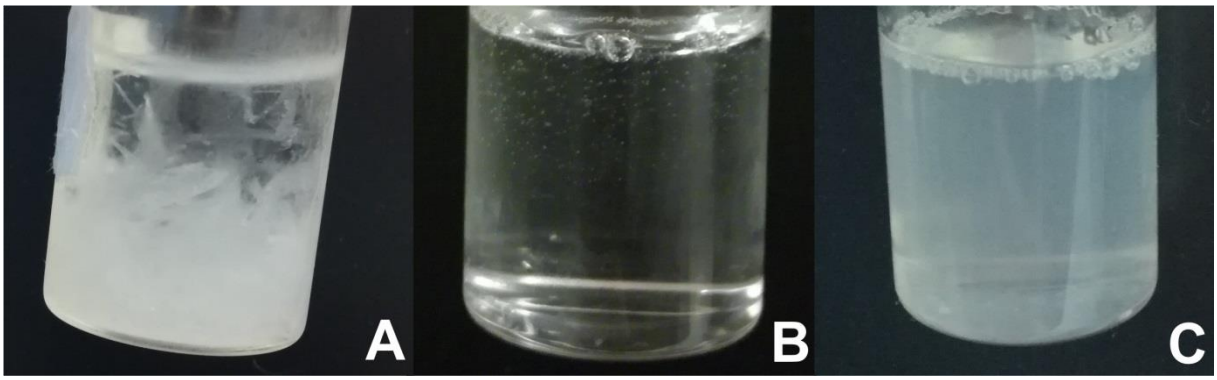

Figure 22. Comparison between different stages of preparation of solution

4.2.6 Mechanism of solubility of chitin in green solvent

Figure 23 shows the proposed mechanism of dissolution of chitin in pre-cooled urea/sodium hydroxide aqueous solution. At first, the complex was soaked in the solvent (1) at low temperature for a short time period. The water molecules entered between the chitin molecules assisted by sodium hydroxide molecules (2). Freezing and expansion of the water at the freezing point broke inter/intra hydrogen bonds between the chitin chains (3), thus promoting the solubility of the chitin (4). However, above -15 °C, the freezing expansion process was shorter, and the expanding effect weakened reducing the chitin solubility.

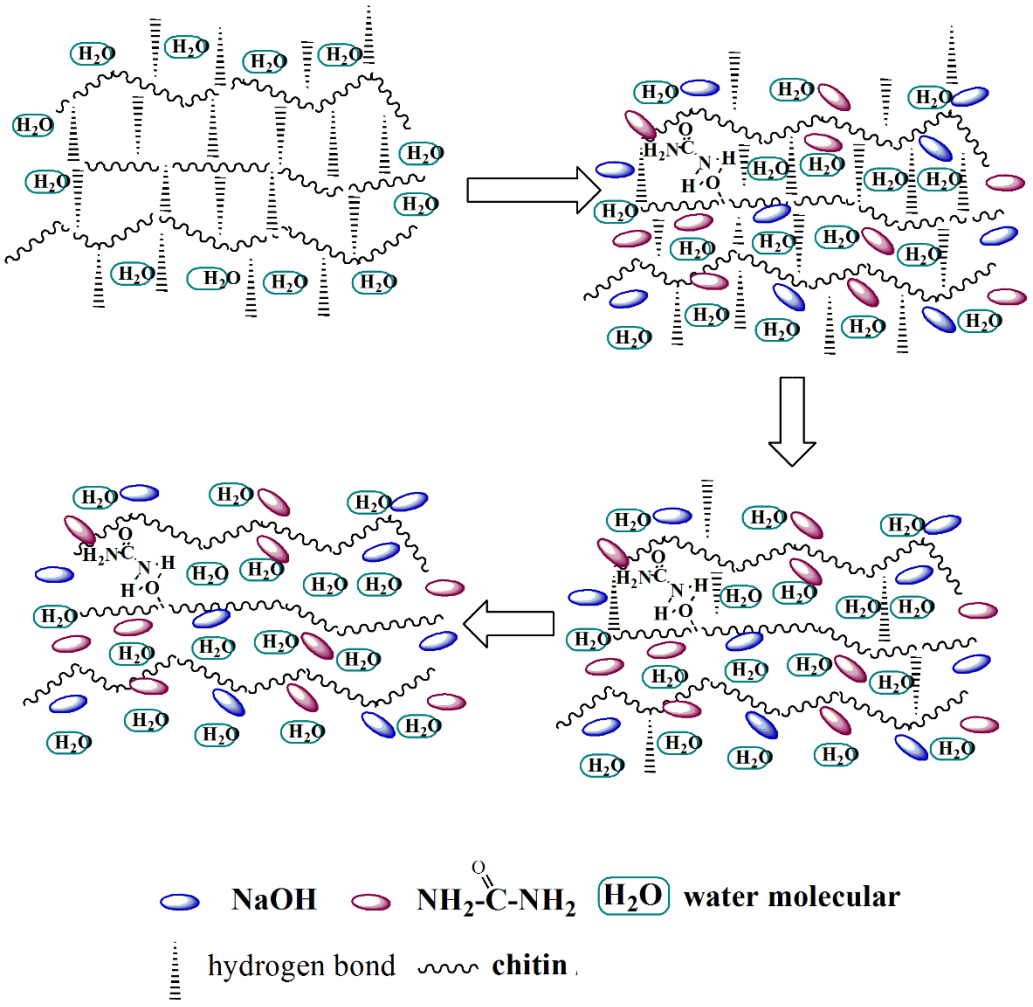

Figure 23. Illustration of the dissolution process of chitin; chitin soaked in 12 wt.% NaOH/8 wt.% urea aqueous solution at room temperature; (1) water molecules enter into chitin molecular chain facilitated by the NaOH; (2) water molecules freeze and expand at the freezing temperature, and break the inter-and intra-hydrogen bond; (3) promoting solubility of the chitin (4).

4.3 Rheology of chitin solutions and chitin/PVA mixtures

4.3.1 Rheology of chitin solutions

The characteristic properties of prepared solutions have been studied by rheological measurements. The aim of these measurements has been study of elastic and viscous behaviour of the samples, their mechanical properties and viscosity. The flow curves are showed in figure 24. In this figure can be seen dependence of dynamic viscosity on shear rate of chitin solutions in concentration range from 0,5–4 %. On the flow curves is visible the trend of increasing viscosity with chitin concentration in samples. With increasing concentration, the flow properties of the samples also changed. At lower concentration of chitin, the solutions behaved like Newtonian fluids and thus their viscosity is not dependent on shear rate and it has been constant during whole measurement. At higher chitin concentrations, the solutions began to behave as non-Newtonian, and their viscosity slightly changes as the shear rate increases.

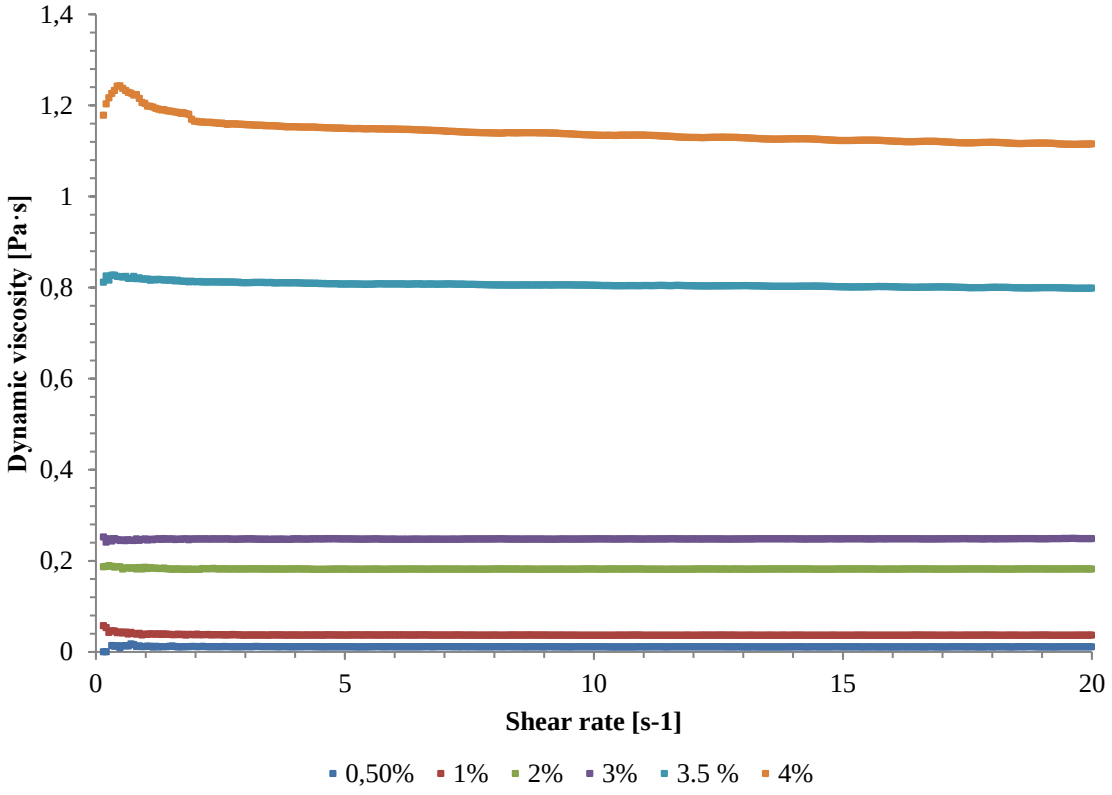

Figure 24. The flow curves of chitin solutions.

As a part of this work was also studied the dependence of solution properties on time. In figure 25, the dependence of the dynamic viscosity on the shear rate of two 3 % chitin solutions is shown graphically. On the displayed dependence we can see the difference in viscosity of the two solutions with the same concentration of chitin. The first solution was stored for 1 month in the freezer before the measurement. The second solution was measured immediately after the solution was stable. The difference in viscosity is due to a change in internal structure of the solution over time. This change could be caused by hydrolysis with 12 % of sodium hydroxide solution used to prepare the solution.

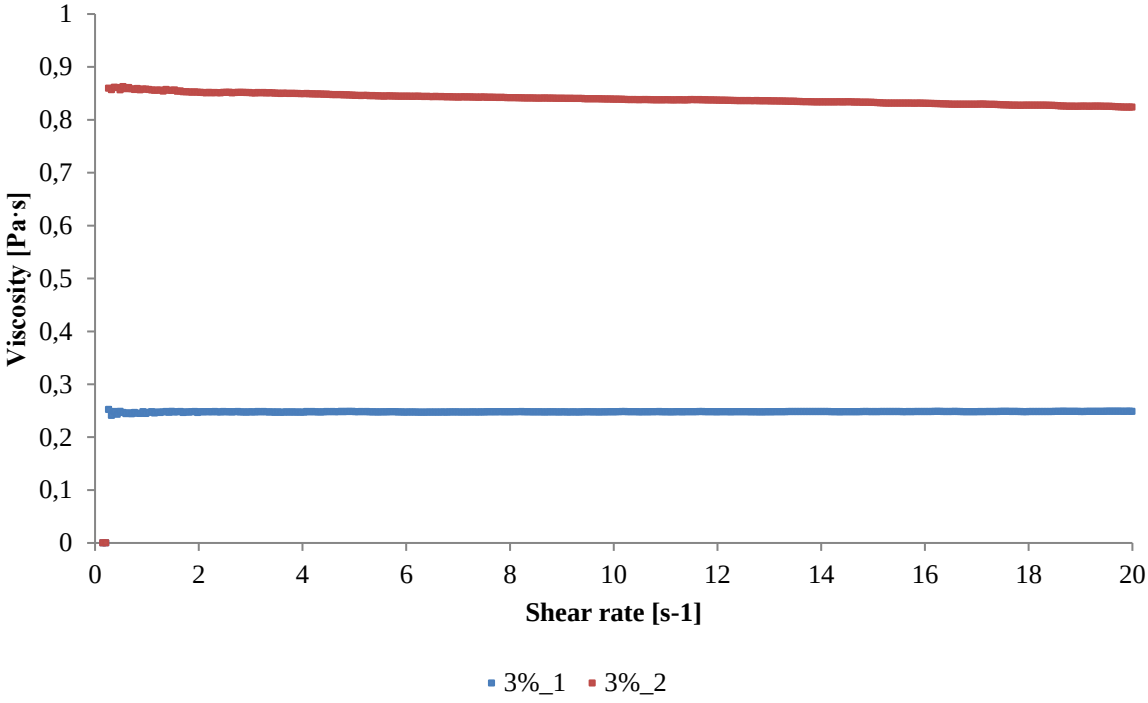

Figure 25. The flow curves of 3 % chitin solutions, the sample 1 was stored in the freezer for one month, the sample 2 was measured immediately after preparation.

To study viscosity changes with increasing temperature was used temperature ramp in the range from 5 °C to 60 °C. In the figure 26 are shown dependencies of dynamic viscosities on temperature for all prepared samples.

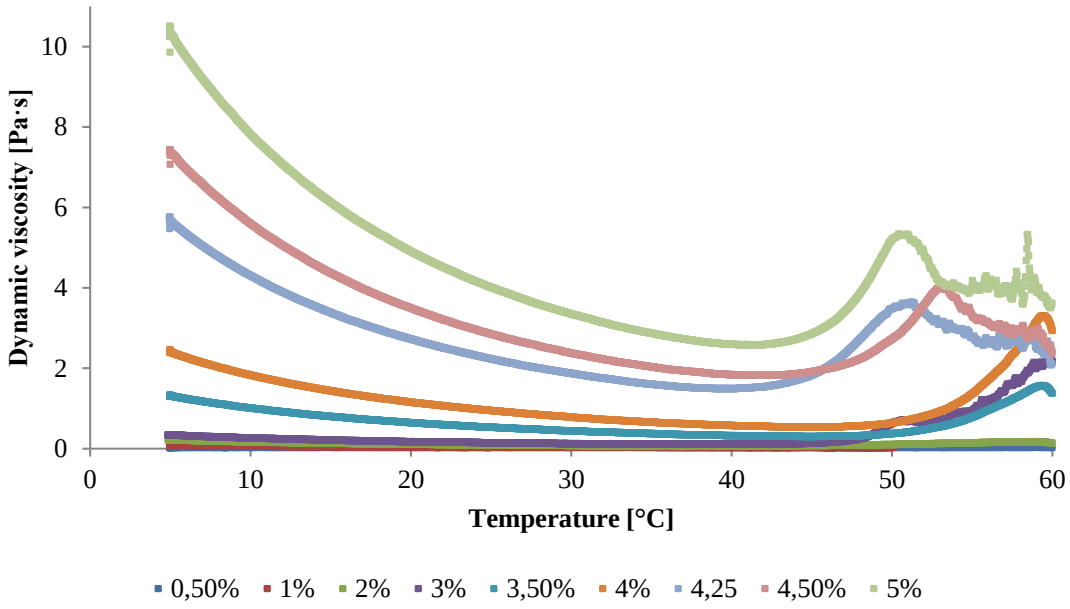

Figure 26. Temperature dependence of viscosity of all prepared samples.

Since on the previous graph is hard to read, three representative concentrations have been selected and their dependencies are shown on figure 27. On the graphical representation can

be seen that the viscosities started to increase at the 50 °C. This trend may be due to the gelling of the sample or its evaporation as the geometry used for the measurement did not allow to prevent evaporation.

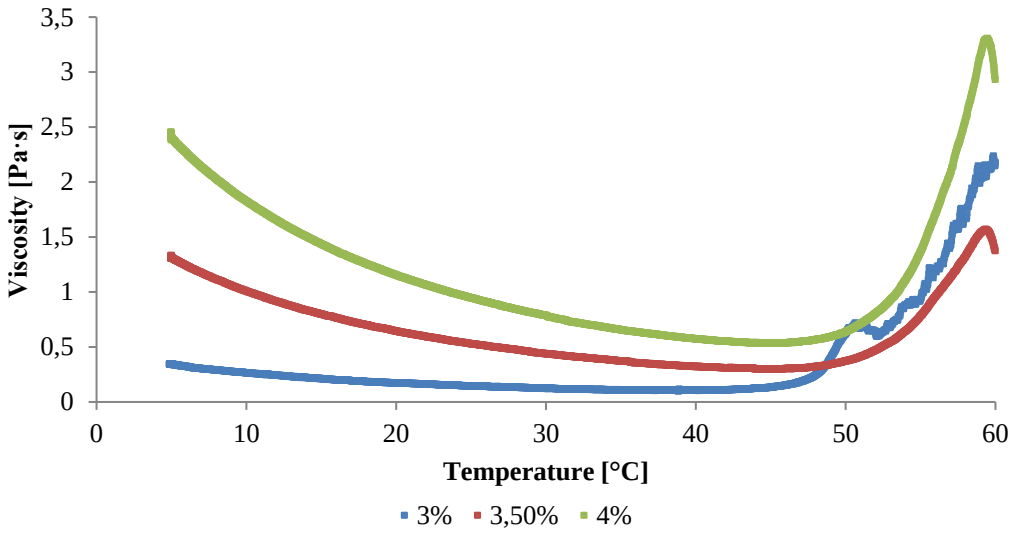

Figure 27. Temperature dependence of viscosity of chosen concentrations of chitin solutions.

To determine if the change in viscosity was actually gelling or evaporation, an oscillating measurement was performed with a change in temperature and tracking storage and loss modulus dependency. The moment when storage and loss modulus curves cross is called gel point. In the figure 28, can be seen the dependency of storage and loss modulus on temperature. The cross-linking occurs at 48 °C, which can be considered as the gelation point of 3 % chitin solution. On the graph is shown one selected area from measurement to see the cross point.

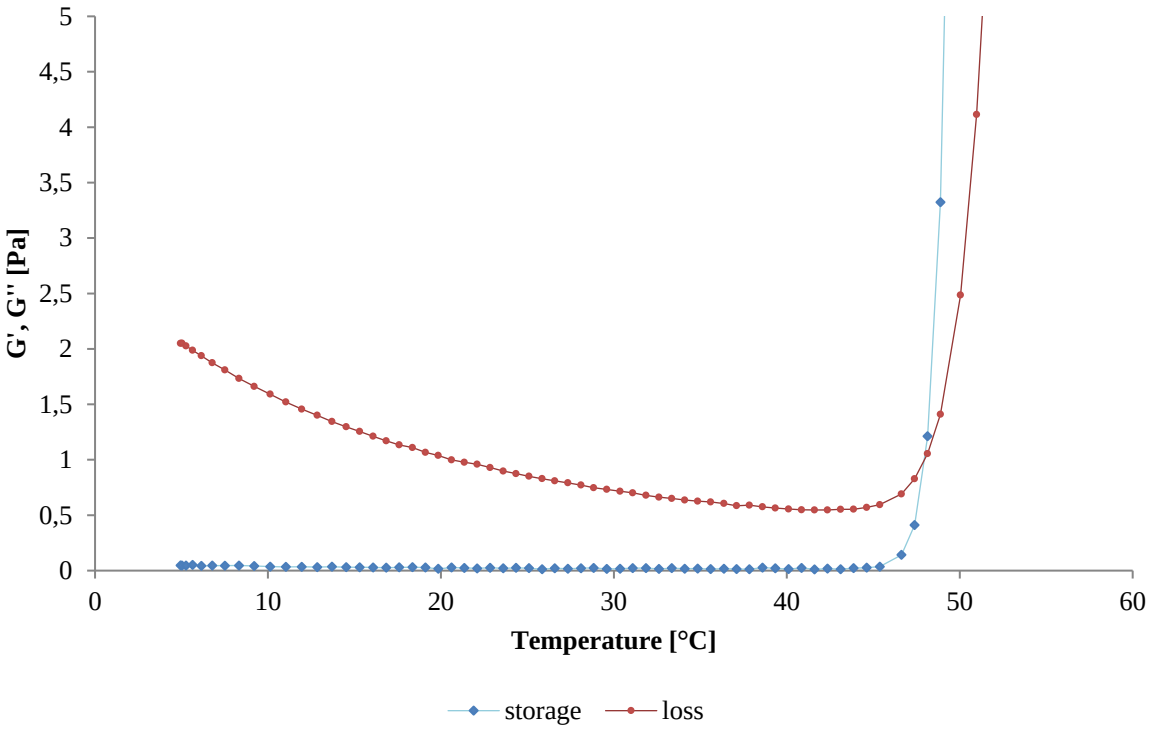

Figure 28. Temperature dependence of the storage modulus G’ and loss modulus G’’ for 3 % chitin solution.

In the figure 29 can be seen dependency of storage and loss modulus of solutions with different concentrations. In the figure 30 can be seen two selected concentrations. When the curves of the storage and loss modulus cross, this point is called cross point. If the cross point occurs at higher angular frequency the system is stronger. The storage and loss modulus were measured at low angular frequency, the maximum was 20 Hz. In the range of 0,1–20 Hz the cross point did not occur. However, for all solutions, it can be said that the storage and loss modulus are approaching and thus can be assumed that the cross point occurs at a higher angular frequency.

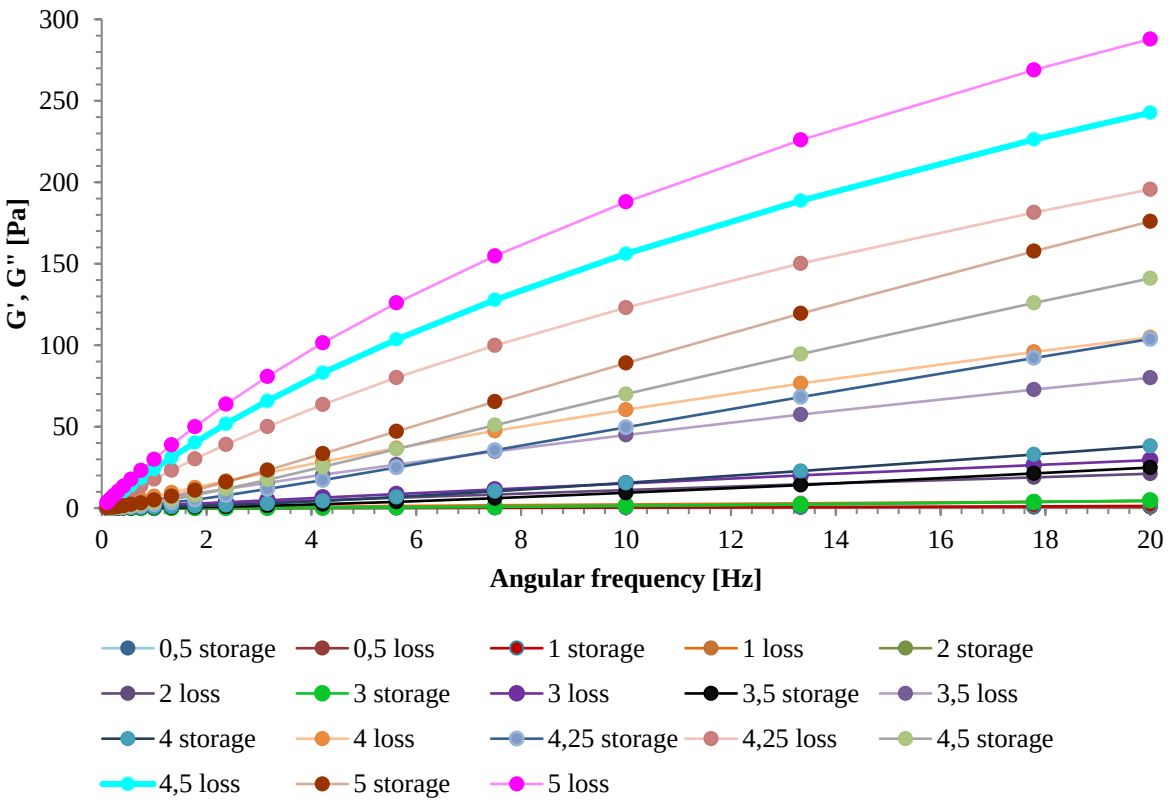

Figure 29. The dependency of storage and loss modulus on angular frequency of all prepared chitin solutions.

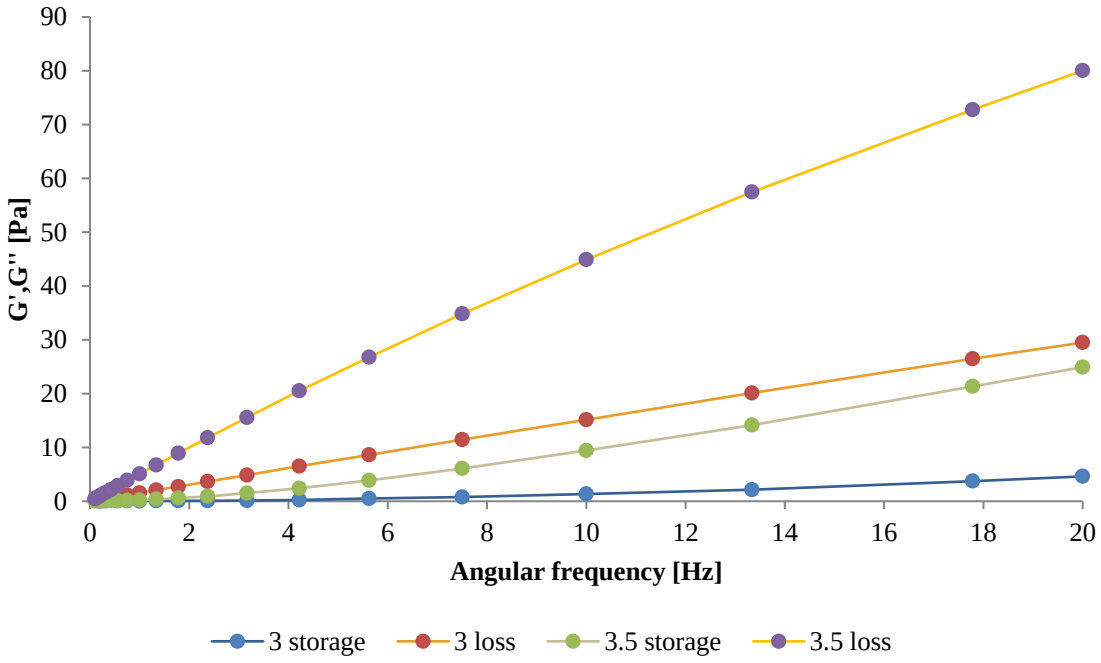

Figure 30. The dependence of storage and loss modulus on angular frequency for chosen 3 % and 3,5 % concentrations.

4.3.2 Rheology of PVA/chitin mixtures

As in the previous chapter, the flow test was performed on prepared solutions of PVA and chitin. The flow curves are showed in figure 31. It can be seen that all solutions act like Newtonian fluids. The viscosity of the samples decreases with increasing the amount of PVA in the solutions.

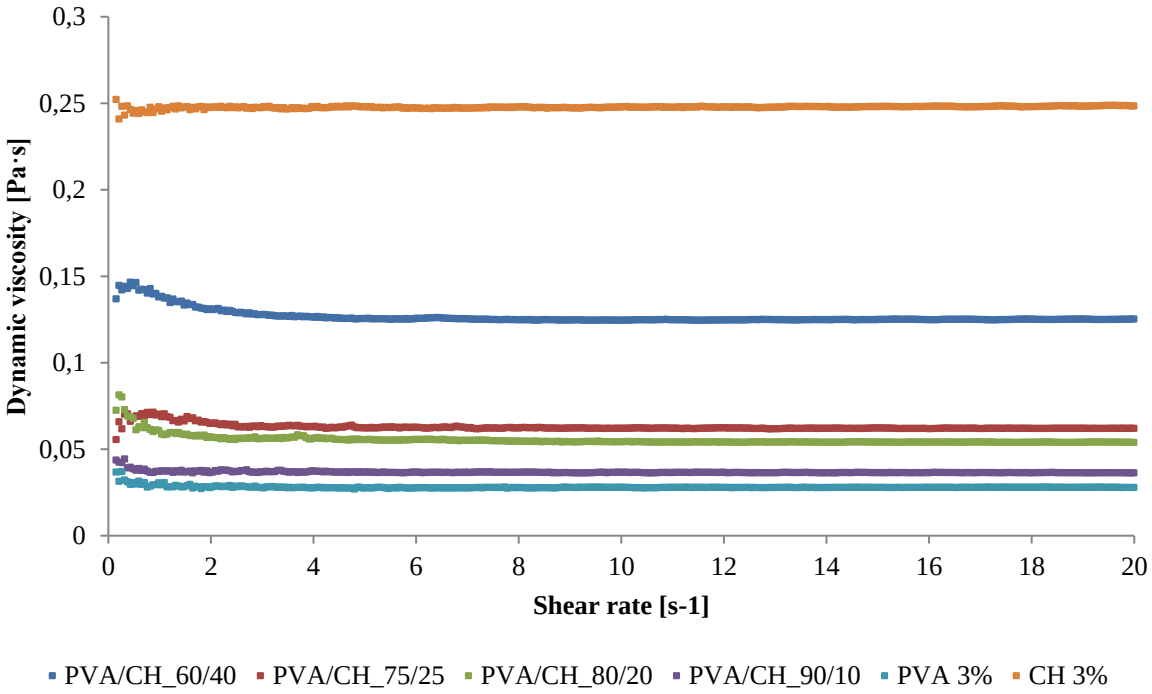

Figure 31. The dynamic viscosity dependence on shear rate of all prepared PVA and chitin mixtures

In the figure 32 can be seen the dependency of storage and loss modulus on the angular frequency. During all the measurement the loss modulus of all solutions was higher than the storage modulus. If the loss modulus is higher than storage modulus that means that samples report more viscous behaviour, that act like a fluid. In the figure 33 can be seen selected sample, used to make films.

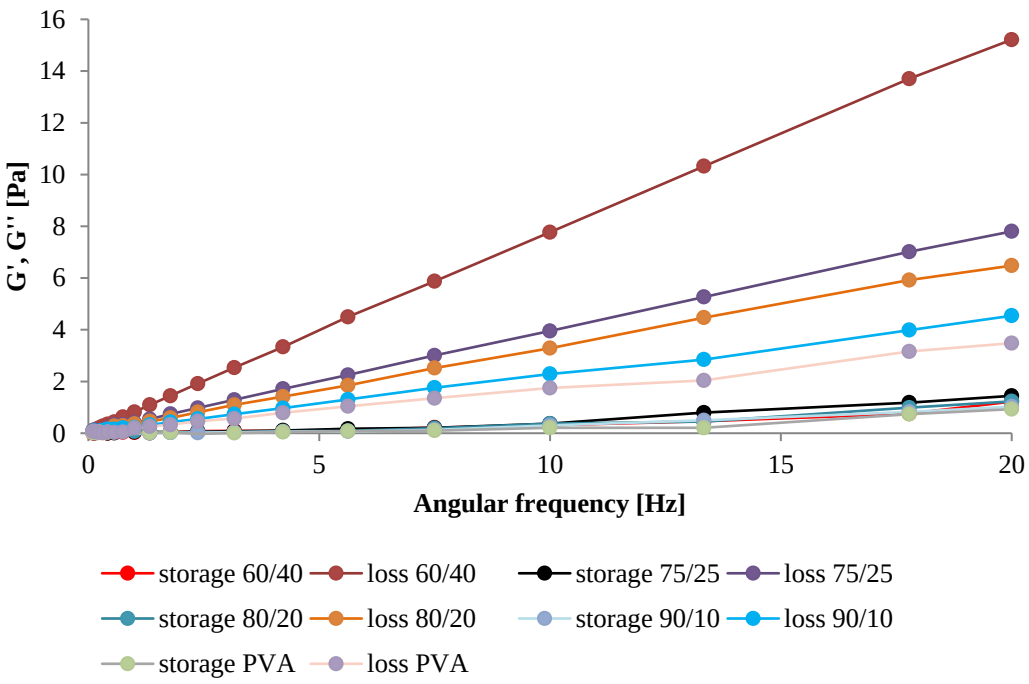

Figure 32. The angular frequency dependence of storage modulus G' and loss modulus G'' of prepared mixtures of PVA and chitin.

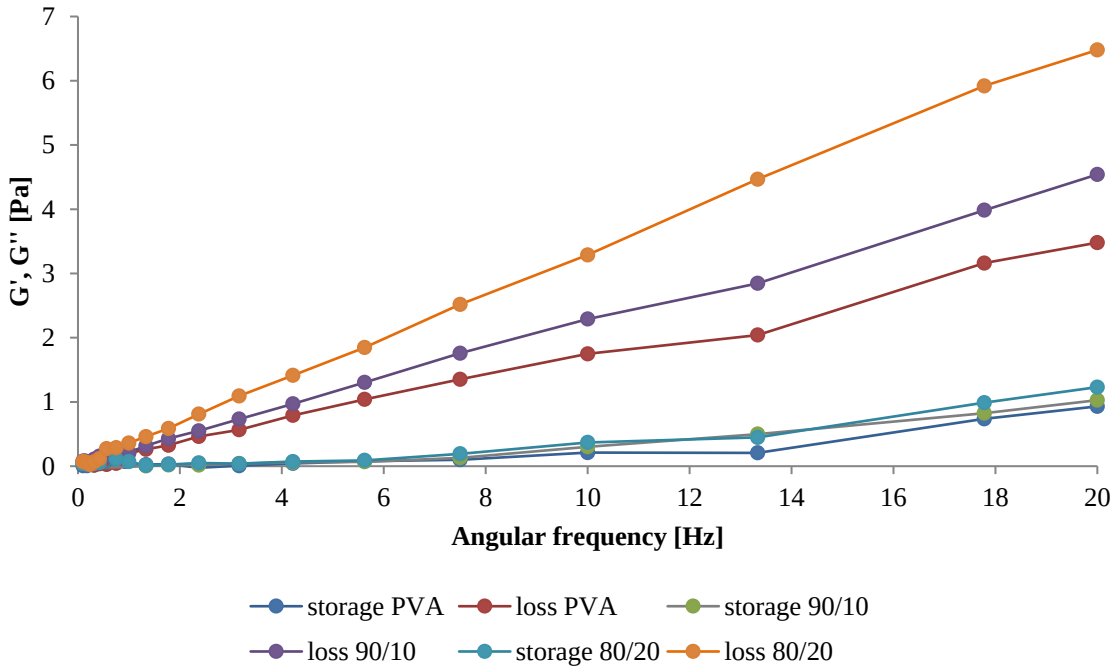

Figure 33. The angular frequency dependence of storage modulus G' and loss modulus G'' of chosen samples.

4.4 Preparation of film

The preparation of 3 % chitin film was successful and comparison between the solution and dried film can be seen on the figure 34.

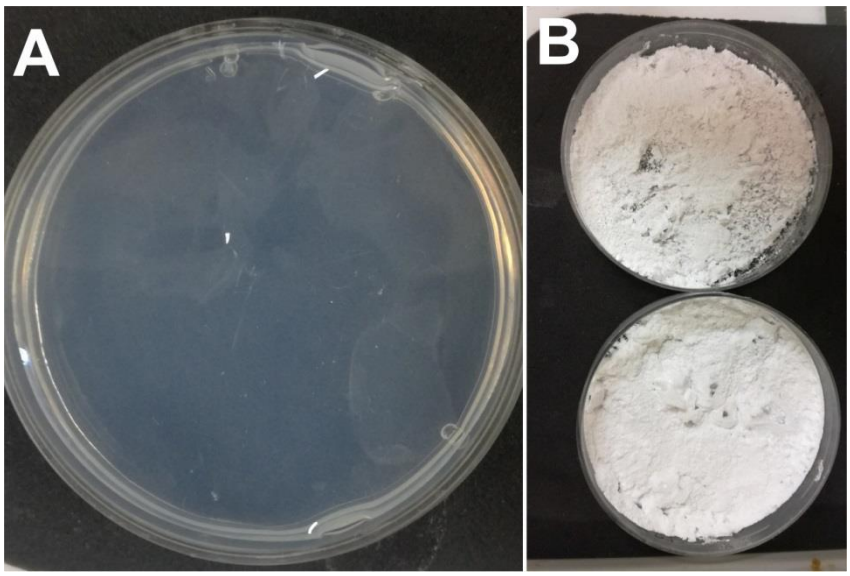

Figure 34. Chitin films. The picture A shows chitin solution after placing it on the Petri´s dish and picture B shows how it looked after drying.

Films were prepared from chitin and PVA mixture, both of them of concentrations 3 %. After air-drying, salts appeared on the surface of the films, which were subsequently removed by washing in distilled water. The 3 cycles of washing were required to wash all salts from the surface. After washing the films were dried in the oven for 96 hours. The films shrink by about 30 % during drying.

The film of mixture of PVA/CH in ratio 60/40, was not prepared. It was obtained non-compact structure which was destroyed by the action of water during washing.

The films with a higher concentration of PVA could not been obtained transparently by despite care washing. The edges of the films were transparent and there was a strong white haze in the centre, which can be seen on figure 35 and figure 36. For characterization of films were chosen three of them, the pure PVA which is shown in figure 37, the PVA/CH_90/10 and PVA/CH_80/20. The TGA and FT-IR characterization was also done for PVA/CH_75/25.

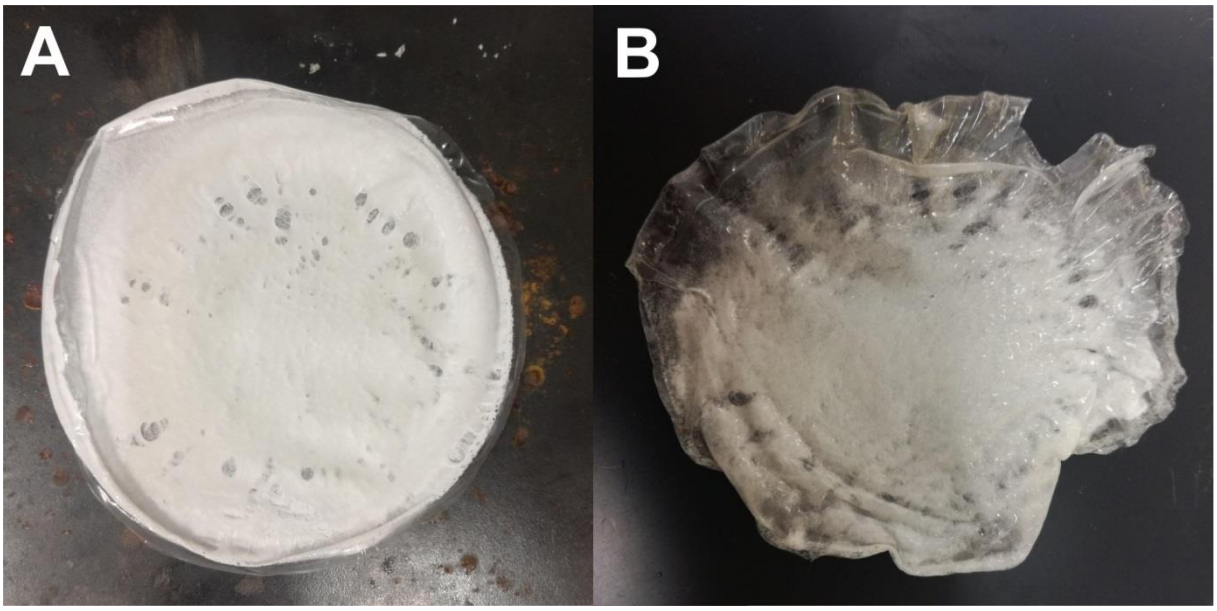

Figure 35. The PVA/CH film with concentration 90/10. Picture A shows the film after air-drying and picture B shows the film after washing and drying in oven.

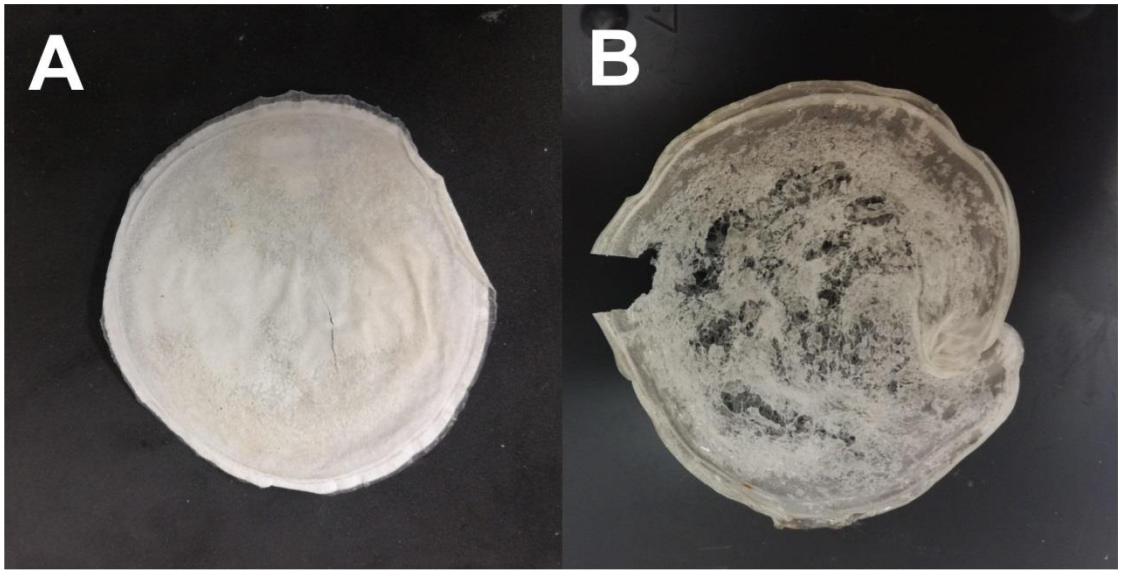

Figure 36. The PVA/CH films of different concentration after washing and drying. The picture A shows a film with concentration 75/25 and picture B shows a film of concentration 80/20.

Figure 37. The film of net PVA.

4.5 Characterization of films

4.5.1 FT-IR

In the figure 38 can be seen FT-IR (ATR) spectrums of all prepared films. The films showed characteristic peaks of PVA at 3 364 cm^{-1} which corresponds to O-H group, the peak at 2 943 cm^{-1} corresponds to -CH_2 group. The characteristic peak at 1 262 cm^{-1} corresponds to C-O stretching and 1 097 cm^{-1} corresponds to C-O-C group. As can be seen on the figure, intensity of these peaks changed with increasing of concentration of chitin in the films. On the spectrums there is a visible trend in decreasing intensity of characteristic PVA peaks with increasing chitin content. The spectrum of PVA containing 25 % of chitin is more similar to that of 3 % chitin film than the one of net PVA.

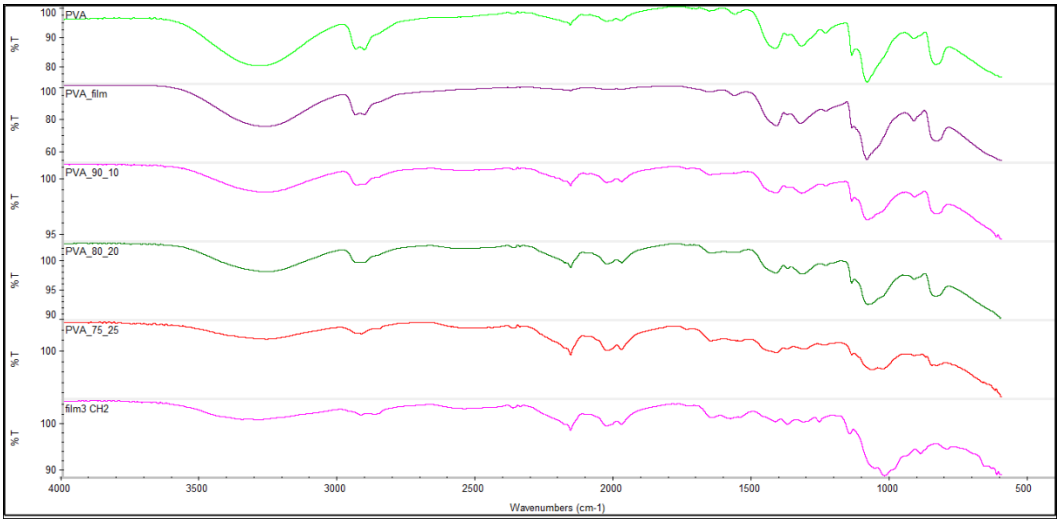

Figure 38. FTIR spectra of all prepared films and PVA powder

4.5.2 TGA

The all thermogravimetric data were recorded under condition mentioned in chap. 3.3.4. On the figure 39 can be seen the thermograms of all prepared films, net PVA and chitin. The PVA shows two stages of degradation. The first stage of decomposition occurred in the range from 248–325 °C (521,15–598,15 K) due to the degradation of PVA. The second stage occurred in the range from 413,26–474,11 °C (686,41–747,26 K). The degradation of PVA/CH films occurred in three stages. The first stage occurred in the range from 80–160 °C (353,15–433,15 K) due to the water absorption of prepared films. The other two steps of degradation are described before. It can be seen that the thermal decreases with increasing the concentration of PVA in the films. Mayor thermal stability showed the 3 % chitin film.

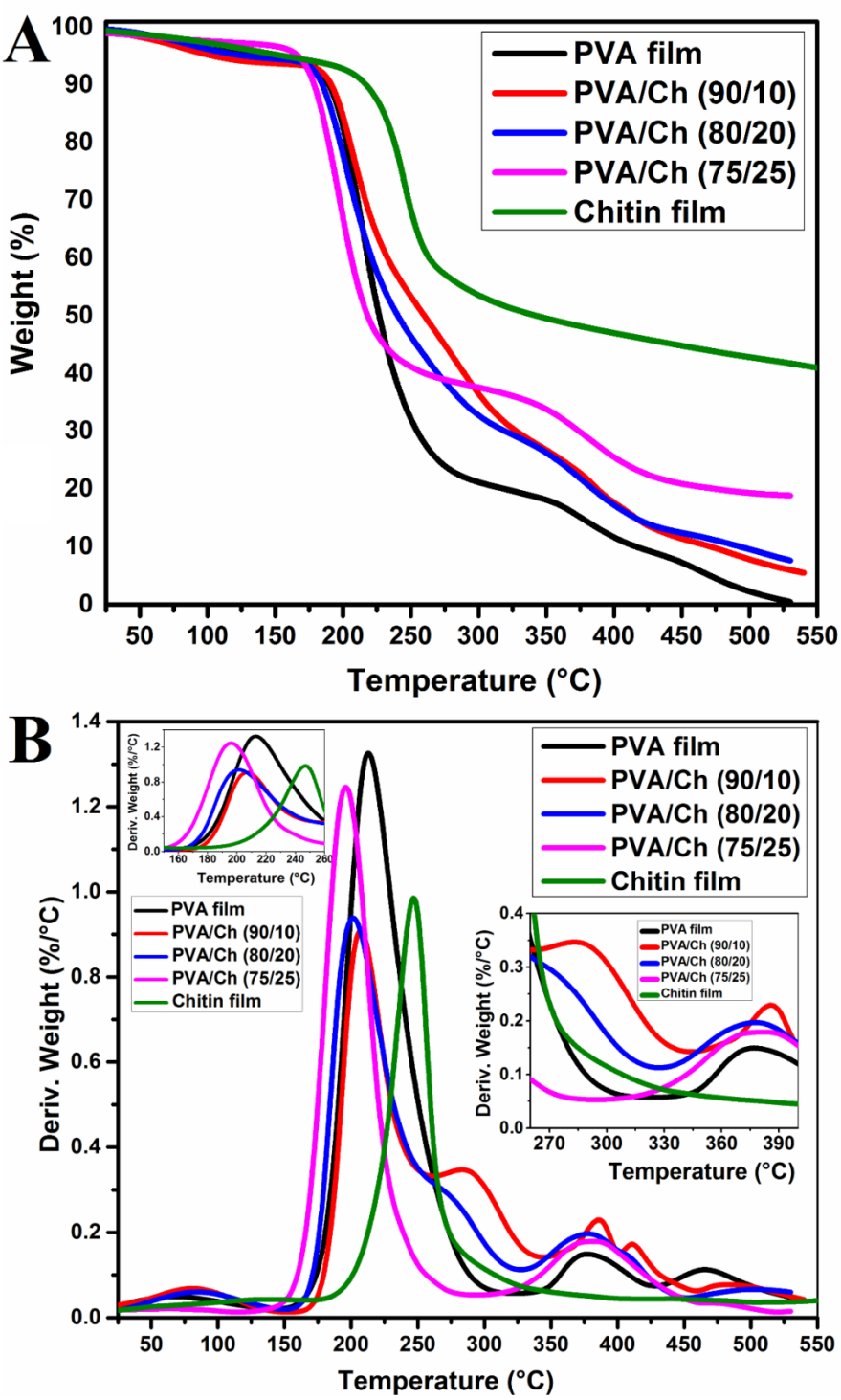

Figure 39. TGA curve of all prepared films, net PVA and chitin

The results of thermogravimetric analysis are shown in table 4. The table shows that chitin has a higher thermal stability than PVA. The all prepared films showed the similar trend in decomposition as net PVA and PVA film. The maximum degradation temperature of net PVA powder was about 14 °C higher than the maximum degradation temperature of the films. Chitin showed the highest decomposition rate at T_{max}, but the residue content of chitin at 500 °C was about 19,33 %. The residue content of PVA films at 500 °C was about 12 % for

the films with 90 % and 80 % of PVA and about 21 % of film with 75 % of PVA. The residue content of net PVA was about 8 % and the residue content of PVA film was about 7 %.

Table 4. Results of thermogravimetric analysis of the films

Sample	Weight loss at Tmax [%]	Maximum degradation temperature Tmax [°C]	Decomposition rate at Tmax [%/°C]
PVA	68,98	271,36	1,400
CH	53,37	368,90	1,532
PVA_film	77,06	257,74	1,066
PVA/CH_90/10	74,18	255,04	0,992
PVA/CH_80/20	70,68	257,77	0,982
PVA/CH_75/25	54,17	255,30	1,326

4.5.3 SEM

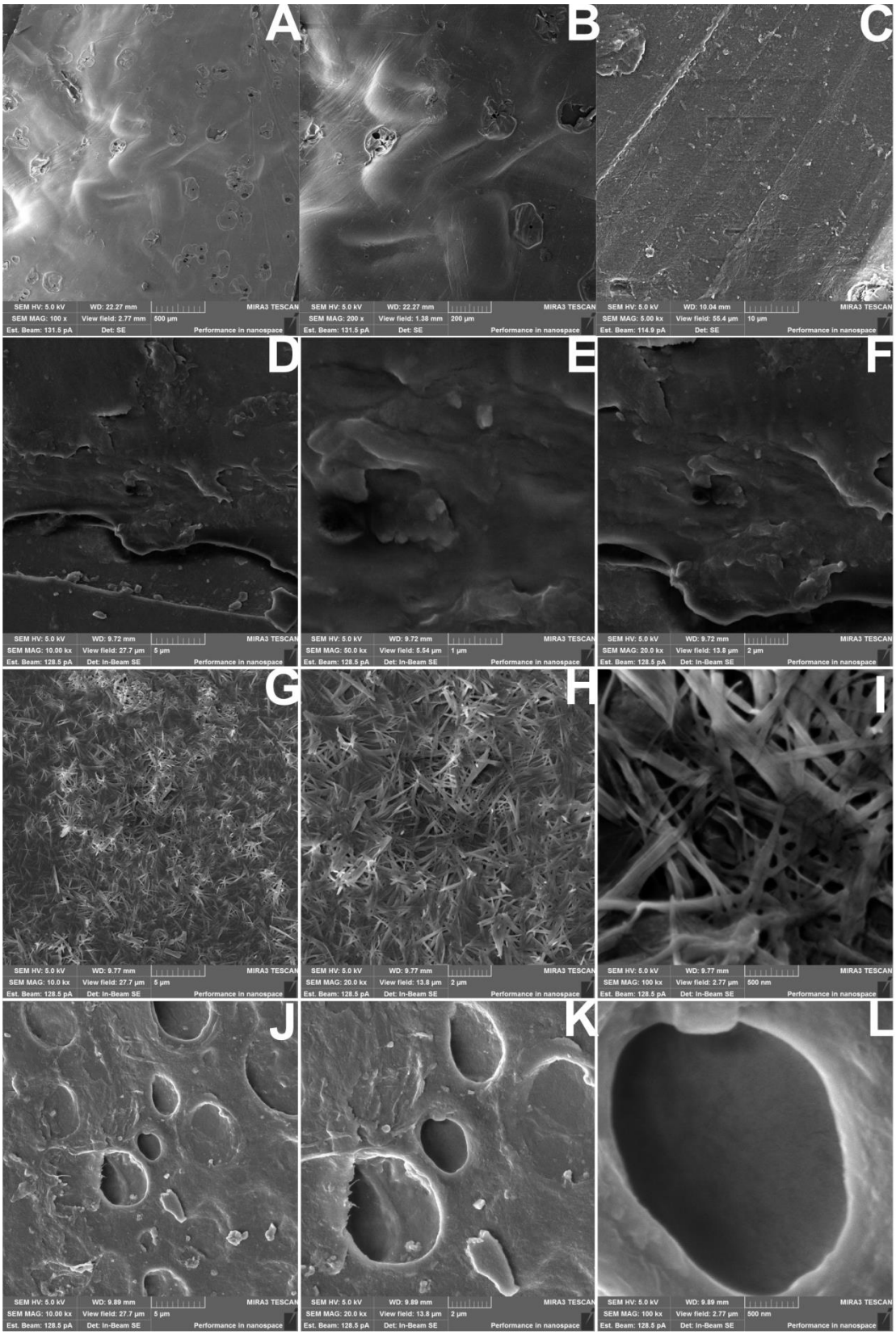

Figure 40. SEM photographs of prepared film. Net chitin film (A-C). Net PVA film (D-F). (G-I) surface morphology of PVA/chitin film with ratio 90/10. (J-L) surface morphology of PVA/ chitin film with ratio 80/20.

The SEM pictures of net chitin (A–C) show many interruptions in the film structure. The hole structure corresponds to removal the salts from the surface with deionized water and ethanol, or might be due to small bubbles in viscous chitin solution that cannot be removed by centrifuge. SEM pictures of net PVA (D–F) with different magnifications show that this film has a compact structure organized into layers sticked together. In the picture F can be seen that the film of PVA have more roughness surface compared to net chitin film. Figure 40 (G–I) shows SEM photographs of PVA/CH (90/10) that shows that the entire sample surface is covered with crystal salts of sodium chloride (flower form). These particles are elongated up to 5 μm long and create fibrous like texture. Particles are needle shaped, straight and rarely slightly curved. They do not have preferred orientation but form feathery clusters. The entire texture form surface layer and singular particles tend not to stick up from the layer. This might be due to the film was not enough washed with water and still have some sodium chloride on the surface of blended film. SEM pictures of PVA/CH 80/20 (J–L) shows that in the surface of the film there are considerable interruptions in the structure. These interruptions are likely to have occurred in the removal of salts from the surface or in the preparation of the sample for measurement and may have caused the mechanical properties deterioration. Figure 41 shows the SEM of chitin, PVA nets and film with different ratios between PVA and chitin after diffractive deformation. As shown in figure 41A, chitin film shows compact structure without any holes between chitin layers. In figure 41B of net PVA it can be seen small crack appeared in cross-section of PVA. In figure 41 (B, C) shows plastic deformation of the blended film with some fibrils shape.

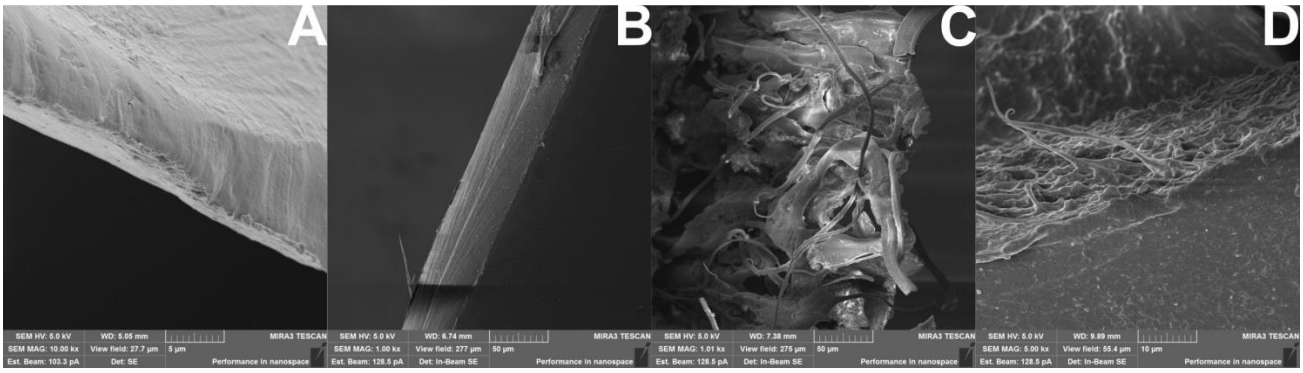

Figure 41. Cross-section of all prepared films. A- net chitin film, B-net PVA film, C-PVA/CH_90/10, D- PVA/CH_80/20.

4.5.4 XRD

Figure 42 shows the X-ray diffraction of net chitin, PVA and film with different ratio between PVA and chitin solution. Pure chitin film shows different peaks at 2θ= 9.4º, 12.9º, 19.3º, 23.5º, 26.5º index as (020), (021), (110), (120) and (013) respectively. Suggesting the crystallinity of α-chitin after purification. Pure PVA film showed only one peak at 2θ= 19.8º and one broad peak to 2θ= 11-13º, In PVA/chitin film showed different peaks corresponded to PVA and chitin polymer with lower intensity compared with net chitin. Comparing between net chitin and regenerated chitin film, there is no significant difference between net and generated chitin film. These results further demonstrated that the chitin dissolution and regeneration of chitin film are physical process and no chemical reaction occurred.

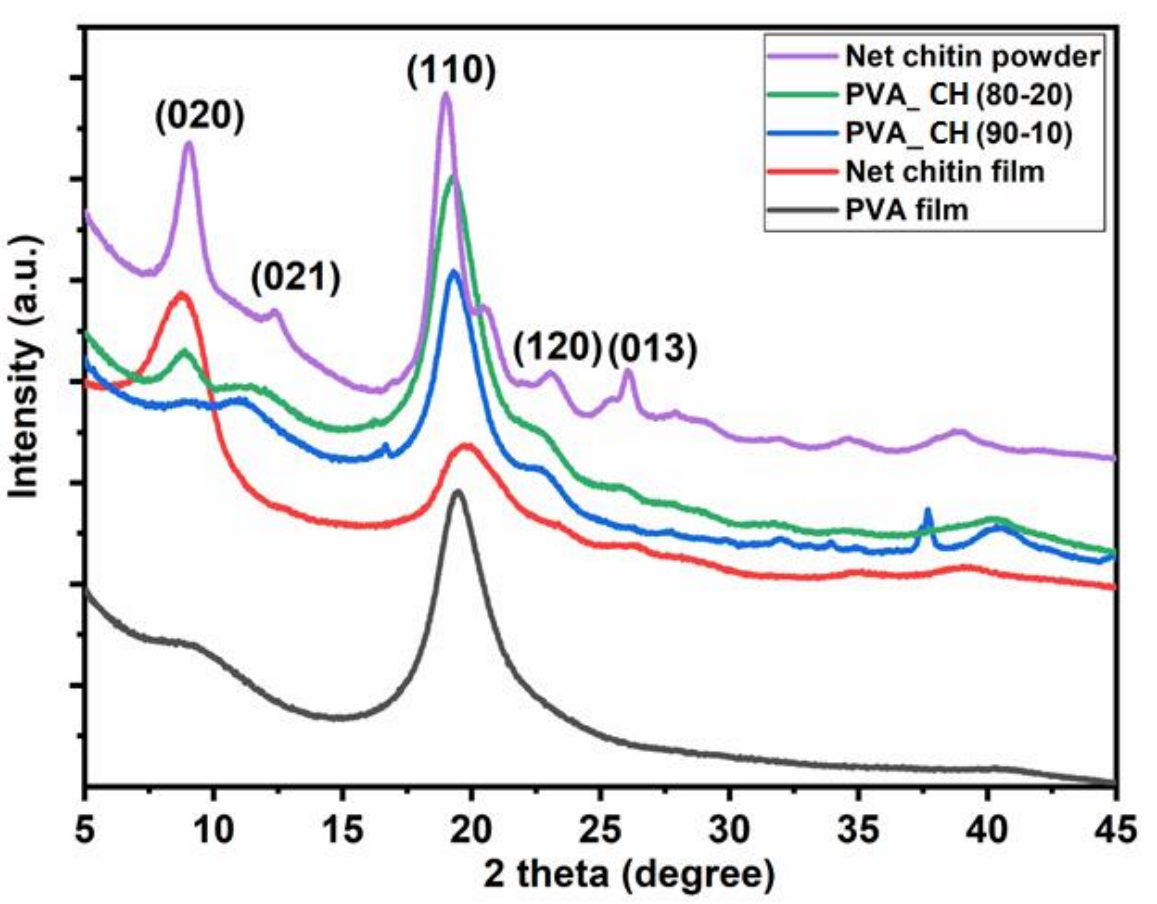

Figure 42: Representative X-ray diffraction of composite film (PVA-chitin) compared with PVA film and net chitin.

4.5.5 Mechanical tests

The tensile test of PVA/CH films has been performed to study mechanical properties of the films. The tensile test has been performed on selected films on net PVA film, on the film with concentration of PVA 90 % and of chitin 10 % and on the film with concentration of PVA 80 % and of chitin 20 %. The specimens that were used for measurement are shown in figure 43. In figure 44 can be seen the dependency of standard force on applied strain. It can be considered that the mechanical properties are getting worse by increasing the amount of chitin in films. The results of the measurement are shown in table 5.

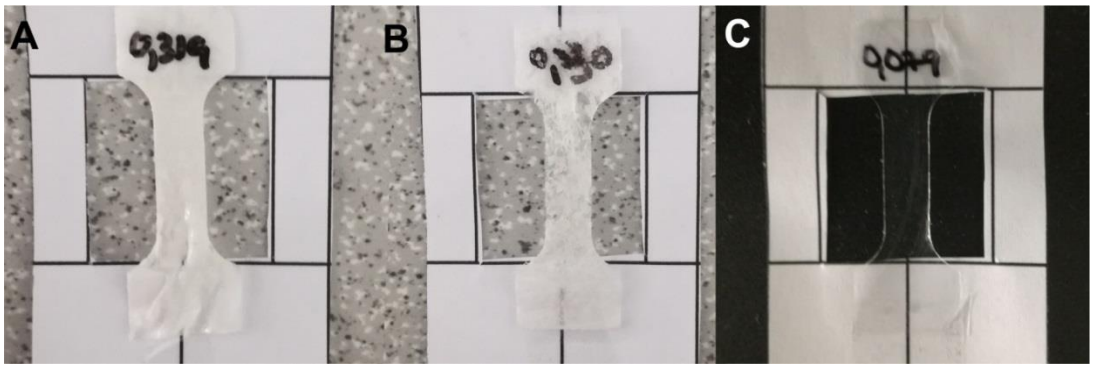

Figure 43. The specimens used for mechanical tests

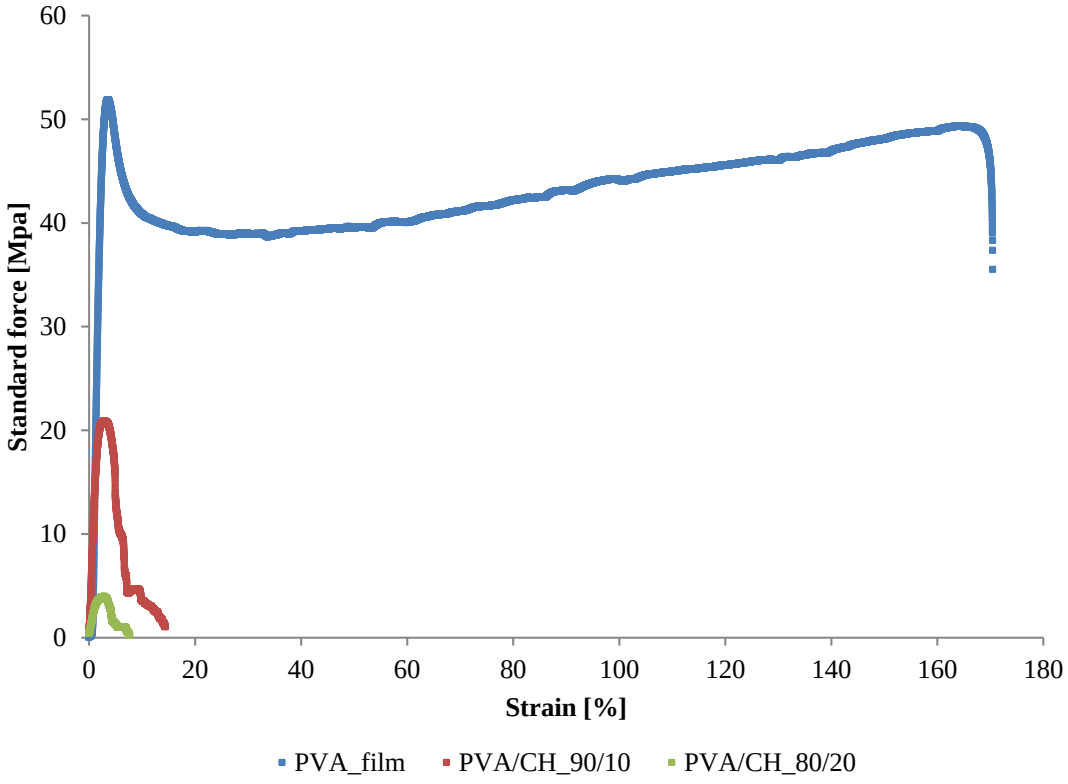

Figure 44. Dependency of the standard force on strain.

Table 5. The results from tensile tests.

	E_t [GPa]	σ_Y [MPa]	σ_M [MPa]	ε_M [%]	ε_B [%]
PVA_film	3,37 ± 0,4	44,34 ± 5	49,86 ± 2	175,26 ± 9	183,42 ± 10
PVA/CH_90/10	1,00 ± 0,005	20,28 ± 0,6	20,28 ± 0,6	3,18 ± 0,4	5,02 ± 0,5
PVA/CH_80/20	0,25 ± 0,05	3,44 ± 0,5	3,44 ± 0,5	2,65 ± 0,13	6,95 ± 1,4

5 Conclusion

Pure chitin fibrils were extracted from shrimp shells by acid and base treatment with control the degree of deacetylation (DDA). Pure chitin with very low residual of protein less than 0,05 % and with 9 % DDA was obtained after 10 h of acid treatment using 5 % sodium hydroxide (48 h, 90 °C). Pure chitin was characterized by different techniques like acid-base titration, FTIR, TGA, XRD, solid NMR, and SEM. Green solvent-based urea/sodium hydroxide was used for dissolution chitin fibrils obtained different concentrations of chitin solution (0,5 to 5 %). The rheological properties of the different chitin solution were measured and evaluated and 3 % of chitin solution was used for preparation of chitin film. PVA/chitin films were prepared by casting method using different ratio between chitin and PVA. The mechanical properties, rheological properties, XRD, TGA of film were measured and evaluated.

The chitin solutions were characterized by rheological measurements. The temperature dependence of dynamic viscosities of these solutions was measured. The viscosities of solutions of concentrations higher than 3 % increased after reaching 50 °C. For the 3 % chitin solution selected for film preparation, the dependence of the storage and loss modulus on temperature was measured wherein during this measurement the gelation point occurred. During frequency sweep measurement the cross-point did not occur, but based on graphical dependencies, it can be assumed that crossing occurs at higher angular frequency. The films were prepared from blended solutions of (3 %) PVA and (3 %) chitin solution. The blended films with different ratios between PVA and chitin (90/10; 80/20) were prepared and used for tensile tests, FT-IR, TGA, SEM and X-RD. The film with concentration 60 % of PVA and 40 % of chitin was not stable and compact and disintegrated by the action of water. The TGA showed that the increasing amount of PVA thermostability of prepared films slightly decreases. Tensile tests performed on the three selected films showed that the mechanical properties deteriorated with increasing chitin concentration.

Preparing chitin solution and films is a lengthy and complex process. The prepared films do not show sufficient mechanical properties and compactness. Film improvement as well as more detailed characterization of individual solutions will be subject to further research.

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7 List of used short cuts

DA- degree of acetylation

DDA- degree of deacetylation

NMR- nuclear magnetic resonance

FT-IR- Fourier-transform infrared spectroscopy

ATR- attenuated total reflectance

HA- hyaluronic acid

PVA- polyvinyl alcohol

CH- chitin

TGA- thermogravimetric analysis

X-RD- X-ray diffraction